18 Acquired marrow failure syndromes: aplastic anemia, paroxysmal nocturnal hemoglobinuria, and myelodysplastic syndromes

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The online version of this chapter contains educational multimedia components on pathogenesis and treatment of PNH and on CHIP, ICUS, CCUS, and MDS and the role of clonal evolution.

Introduction

The bone marrow failure (BMF) syndromes comprise a heterogeneous group of clinically and pathologically distinct disorders associated with cytopenias and failure of normal hematopoiesis. In BMF disorders, the inability of hematopoiesis to meet physiological demands for the production of healthy blood cells can result in either pancytopenia or cytopenias involving specific lineages. The etiology of marrow failure in an individual patient can be multifactorial or related to a single cause. These various disorders may either be extrinsic or intrinsic to the marrow. An example of an extrinsic cause is the inappropriate immune response that results in aplastic anemia, whereas the hematopoietic progenitor or stem cell defects that underlie the myelodysplastic syndromes (MDSs) are intrinsic. BMF syndromes can be acquired or, more rarely, congenital.

The range of molecular mechanisms responsible for congenital marrow failure states is broad, including abnormal DNA damage response (Fanconi anemia [FA]), defective ribogenesis (Diamond-Blackfan anemia [DBA]), abnormal telomere dynamics (telomere biology disorder [TBD]), and altered hematopoietic growth factor receptor/kinase signaling (congenital amegakaryocytic thrombocytopenia). Similar mechanisms may underlie some acquired marrow failure syndromes, such as acquired haploinsufficiency for ribosomal protein RPS14 in MDS associated with chromosome 5q deletion, which parallels heterozygous ribosomal protein mutations observed in DBA.

This chapter focuses on acquired marrow failure syndromes, including aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and MDS. There is also discussion of idiopathic cytopenia of undetermined significance (ICUS), clonal hematopoiesis of indeterminate potential (CHIP), and clonal cytopenia of undetermined significance (CCUS). For discussion of inherited marrow failure syndromes, please refer to Chapter 15.

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Table 18-1 Classification of aplastic anemia by severity

Peripheral blood cytopenias	Nonsevere (moderate) aplastic anemia (not meeting criteria for severe disease)	Severe aplastic anemia (any 2 of 3)	Very severe aplastic anemia (meets criteria for severe disease and absolute neutrophils <200)*
Bone marrow cellularity	<25%	<25%	<25%
Absolute neutrophil count		$<0.5 \times 10^9$/L	$<0.2 \times 10^9$/L
Platelet count		<20,000/μL	
Reticulocyte count		<1.0% corrected or <60,000/μL	

*Very severe aplastic anemia is reserved for patients who fulfill criteria for severe aplastic anemia but with an absolute neutrophil count of $<0.2 \times 10^9$/L.

Aplastic anemia

Definition

Idiopathic AA is a hematopoietic stem cell (HSC) disorder associated with reduced bone marrow cellularity and decreased hematopoiesis. This decreased hematopoiesis may disproportionately affect 1 or 2 cell line lineages in early stages of the disease, but AA is ultimately associated with trilineage hypoplasia. Classification and prognosis in AA are related to the depth of cytopenias in the peripheral blood. Severity drives the therapeutic decisions. Severe AA (SAA) is defined by depression of blood counts involving at least 2 hematopoietic lineages (ie, absolute reticulocyte count $<60 \times 10^9$/L, absolute neutrophil count $<0.5 \times 10^9$/L, or platelet count $<20 \times 10^9$/L) and bone marrow hypocellularity (<30%, excluding lymphocytes). Very severe AA has an absolute neutrophil count of $<0.2 \times 10^9$/L, whereas moderate AA is characterized by depression of blood counts not fulfilling the definition of severe disease (Table 18-1).

Classification

AA may be acquired and idiopathic, or it can arise in the context of an inherited marrow failure syndrome. This distinction carries profound implications for management and treatment. For example, immunosuppression is a therapeutic option in acquired AA, whereas this treatment modality is ineffective in inherited forms of marrow failure. AA is a diagnosis of exclusion, and systemic causes for pancytopenia should be ruled out. The diagnosis of AA usually is reserved for naturally occurring conditions and excludes those patients with a history of cytotoxic chemotherapy or exposure to ionizing radiation.

Epidemiology

AA is primarily a disease of children and younger adults. Another peak in incidence rate occurs in patients 60 years of age and older, although, in these older patients, some reported cases of AA may actually represent hypoplastic MDS. AA is rare in Western Europe and the United States (less than 2 cases per million in the population per year) and more common in Asia, with an incidence rate of 3.9 cases per million per year in Bangkok, 6 cases per million per year in rural areas of Thailand, and 14 cases per million per year in Japan. This increase in Asian AA patients compared with White or multiracial AA patients has been attributed to a genetic disposition (Asian human leukocyte antigen [HLA] type and nucleotide polymorphisms) rather than environmental factors. Both males and females are equally affected. AA can be acquired or constitutional. Idiopathic acquired AA is perceived as a T-cell–mediated autoimmune process with the immune attack at the level of the CD34-positive hematopoietic stem cell. Idiopathic AA is more common than AA associated with toxins, pregnancy, or hepatitis. The association of AA with drug exposure has been of great interest for decades. However, the level of evidence linking AA to specific drugs is variable. The nonsteroidal anti-inflammatory agents indomethacin, diclofenac, and butazones, antithyroid medication (propylthiouracil), certain anticonvulsants (such as hydantoins and carbamazepine), and certain antibiotics such as chloramphenicol and gold salts are more clearly associated with development of AA. Literature shows environmental exposure to benzene is also linked to marrow failure.

Hepatitis-associated AA accounts for 2% to 5% of cases of AA in Europe and 4% to 10% of cases in East Asia. AA has been reported to occur in 28% to 33% of patients requiring orthotopic liver transplantation for fulminant hepatitis. This seronegative hepatitis in patients with posthepatitis AA does not appear to be caused by any of the currently known hepatitis viruses and often is referred to as hepatitis/AA syndrome. An immune pathogenesis following a putative trigger is suspected, but the precise mechanism is unknown. AA evolves with a typical delay of several weeks to months after the episode of hepatitis, usually after the transaminitis has peaked and begins to trend down.

Etiology and pathogenesis

Regardless of the etiology, the hallmark of AA is the reduction in hematopoiesis, as reflected by marrow histology, low numbers of marrow CD34 cells, diminished

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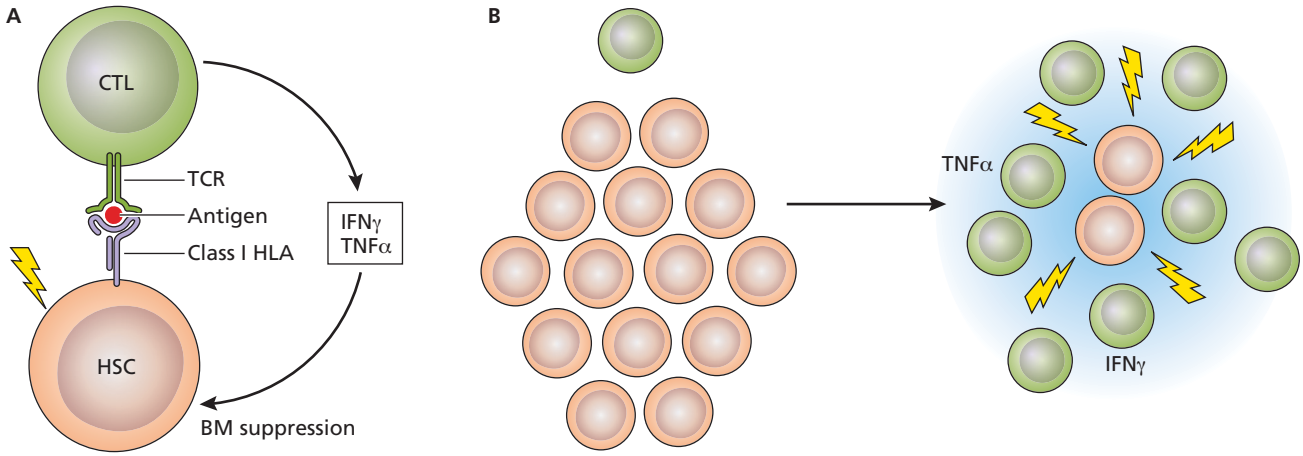

Figure 18-1 Immune-mediated pathogenesis of AA. (A) AA is thought to be initiated by recognition and destruction of HSCs by cytotoxic T cells (CTLs), which recognize some unknown antigen present on the HSCs via their HLA class I molecule. (B) During and/or after immune-mediated BM destruction, a rapid expansion of residual cells (that escaped destruction) occurs, whereby cells carrying mutations achieve clonal dominance and may progress to malignant proliferation. IFN, interferon; TNF, tumor necrosis factor. Adapted from Ogawa S, *Blood*. 2016;128(3):337-347.

numbers of long-term culture-initiating cells (a surrogate measure of hematopoietic stem cells), and poor hematopoietic colony formation in cells obtained from an aplastic marrow. Clinical response to immunosuppressive therapy (IST) targeting T cells (eg, antithymocyte globulin [ATG]), described further in the section on immunosuppressive therapy, supports an immune-mediated pathogenesis of AA. AA is thought to be initiated by recognition and destruction of HSCs by cytotoxic T lymphocytes, which recognize some unknown antigen present on HSCs via their HLA class I molecule. (Figure 18-1A). There are ample data to support this hypothesis, including the presence of T cells at diagnosis that decrease or disappear with IST. Additionally, there is further evidence of increases in proinflammatory cytokines, including interferon γ and tumor necrosis factor α, from aberrantly activated immune cells and stromal microenvironments that also contribute to BMF in AA. This has been attributed to first apoptosis signal (FAS)-mediated apoptosis. Although diverse triggers, such as viruses or chemical hazards, may serve as inciting events in individual cases, the final autoimmune pathway appears to be uniform. It is this pathogenesis and applied IST that may allow for future clonal evolution, discussed in following sections.

Clinical presentation

At presentation, the clinicians should consider the workup shown in Table 18-2. Patients typically have fatigue, weakness, pallor, and headaches resulting from anemia. Often, patients have petechiae of the skin and mucous membranes, epistaxis, and gum bleeding related to severe thrombocytopenia. More severe hemorrhage in the central nervous system or gastrointestinal tract would be atypical at the time of diagnosis. Fever and infections can also be seen in these patients as a consequence of a compromised immune system. Acquired AA patients who are identified early from abnormalities in routine laboratory testing may have no physical manifestations of their disease. AA most often arises in a previously healthy patient who has no history of malignancy and no exposure to cytotoxic drugs or history of radiation exposure. A family history of marrow failure or dysmorphology may help identify inherited causes of pancytopenia. Drug and chemical exposures should be queried in the interview, but these are notoriously difficult to evaluate quantitatively as the history is subject to recall bias. Confirmation of a causal relationship is difficult to ascertain in practice, and management is not likely to differ from those cases without a putative trigger. Discontinuation of a drug strongly suspected to be associated with the onset of pancytopenia is reasonable for a few weeks; however, a prolonged observation period of several weeks to months before initiation of therapy is not recommended, especially when pancytopenia remains severe.

Splenomegaly and hepatomegaly are not typical features of AA and should point toward another diagnosis. Short stature, musculoskeletal abnormalities (particularly radial ray anomalies), dysplastic nails, skin rashes, oral leukoplakia, exocrine pancreatic insufficiency, or other congenital anomalies may suggest an inherited BMF state (see Chapter 15). The absence of characteristic physical findings or a suggestive family history does not rule out an inherited marrow failure syndrome, which can manifest in adulthood with apparent acquired AA or MDS and no physical stigmata. The detection of genetic defects

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Table 18-2 Initial evaluation for presumed aplastic anemia

Patient history	Duration of cytopenias (are previous blood counts known?)
	Medications (prescribed and over-the-counter supplements)
	Exposures
	Transfusions
	Immunodeficiencies or autoimmunity (suspect GATA2)
	Pulmonary fibrosis and/or early onset of lung disease (suspect TBD or GATA2)
	Liver fibrosis, pancreatic insufficiency, or organ anomaly (suspect SDS, TBD, or FA)
Family history	Constitutional abnormalities
	Malignancies
	Other family members with cytopenias
Physical examination	Height (in context of mean parental height)
	Premature graying hair (suspect TBD)
	Limb abnormalities (suspect FA, TBD, DBA, or TAR)
	Skin and nail abnormalities (café au lait spots, nail dystrophy, pale patches) (suspect FA or TBD)
	Organomegaly, lymphadenopathy, or congenital abnormalities
Laboratory	Peripheral blood
	Complete blood count with differential
	Reticulocyte counts, lactate dehydrogenase, haptoglobin
	Chemistries
	Nutritional studies (vitamin B12, folate, iron, copper levels)
	Transaminases and bilirubin
	Infectious serologies (hepatitis, HIV, TB, EBV)
	Beta-HCG (consider even if intercourse is not explicitly stated)
	FLAER flow cytometry assay (PNH)
	Chromosomal breakage tests (diepoxybutane or mitomycin C if FA suspected and/or age ≤40)
	Telomere length and mutational analysis (if TBD suspected, age ≤40 y, or based on clinical picture)
	Bone marrow
	Aspirate and biopsy
	Flow cytometry (including quantitative CD34)
	Cytogenetics
	FISH
	Consideration of gene panel sequencing for inherited BMF or myeloid malignancy based on age ≤40 or based on clinical picture

EBV, Epstein-Barr virus; FLAER, fluorescein-tagged proaerolysin variant; HCG, human chorionic gonadotropin; SDS, Shwachman-Diamond syndrome; TAR, thrombocytopenia with absent radius; TB, tuberculosis.

associated with FA or TBD in some adults with AA but without dysmorphology has blurred the distinction between inherited and acquired forms of marrow failure. It is important to investigate past medical history carefully about earlier blood count abnormalities, macrocytosis, or relevant pulmonary (fibrosis) or liver disease (cirrhosis) as well as review the patient's family while keeping familial or inherited syndromes in the differential diagnosis.

The peripheral blood in AA shows pancytopenia usually with a relative lymphocytosis but is otherwise unremarkable. The bone marrow biopsy in these patients is characterized by hypocellularity. The criteria for the diagnosis of AA (Table 18-1) require either bone marrow with <25% of the normal cellularity or bone marrow with <50% normal cellularity in which less than 30% of the cells are hematopoietic, as the bone marrow in AA can occasionally have increased lymphocytes, which are predominantly mature T cells. In patients with abundant lymphoid infiltrates, immunohistochemical or flow cytometric evaluation may be warranted to rule out an underlying lymphoma. The bone marrow aspirates in AA are correspondingly paucicellular, and the few hematopoietic elements seen do not show overt dysplastic changes. However, erythroid dysplasia alone can be seen in AA and is not diagnostic of MDS. The myeloid cells may show a left shift, but blasts are not increased. Flow cytometric evaluation of the bone marrow in AA is characterized by a relative lymphocytosis. CD34-positive blasts are rare, and the few that are seen show no phenotypic abnormality. If the blasts are phenotypically abnormal or increased, then a diagnosis of hypoplastic MDS should be entertained. Patients with AA may also have small PNH clones; these clones are detected using specialized flow cytometric techniques, as discussed in detail in the PNH section. The identification of a PNH clone may be helpful diagnostically, as PNH clones are not present in inherited or in acquired causes of BMF in younger patients; however, as small PNH clones can also be seen in MDS, this method cannot be used to solely differentiate PNH from MDS in older patients. The presence or absence of a PNH clone is also important to document in AA as its presence may predict a good response to IST. AA is associated with normal cytogenetics. An abnormal karyotype in a patient with a hypocellular bone marrow suggests a diagnosis of hypocellular MDS, although some investigators believe that certain chromosomal abnormalities, such as trisomy 8 or deletion 13q, can still be consistent with an AA diagnosis and not a marker of clonality to define MDS.

Lastly, acquired AA has been associated with telomere length changes. Approximately one third of patients with acquired AA have short telomeres at the time of initial

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presentation. In fact, 10% of patients with acquired AA have mutations in *TERT* (the telomerase gene) or *TERC* (the telomerase RNA template gene), both of which lead to short telomeres. Although the use of telomere length as a treatment response biomarker is still not standard, short telomere length may be predictive of a higher relapse rate and could be a risk factor for clonal evolution.

Differential diagnosis

When evaluating a patient with pancytopenia and a hypocellular marrow, the physician must use appropriate diagnostic workup (Table 18-2) and exclude a number of other conditions before a diagnosis of AA can be made (Table 18-3; Figure 18-2). The most common disorders include MDS, acute leukemia, PNH, an inherited syndrome, certain infections (tuberculosis, HIV), nutritional deficiency (eg, anorexia nervosa), or T-cell large granular lymphocyte (T-LGL) disease (T-LGL populations can coexist with AA or MDS).

The presence of dysplastic immature hematopoietic cells or blast cells should lead to a diagnosis of hypoplastic MDS or acute leukemia. Similarly, marrow cytogenetic analysis may detect a cytogenetic abnormality diagnostic of lymphoid or myeloid leukemic disorders. Hairy cell leukemia frequently presents as pancytopenia with difficulty in aspirating the marrow, or a "dry tap," along with splenomegaly. Pancytopenia can arise in the setting of anorexia nervosa as an epiphenomenon of the eating disorder, possibly because of multiple micronutrient deficiencies. Pancytopenia in this setting is associated with a hypocellular marrow with serous fat atrophy. HIV infection or AIDS is associated with cytopenia, morphologic dysplasia, and marrow hypocellularity in ~10% of cases.

T-LGL is a rare condition characterized by circulating T cells bearing the CD57 marker of effector or cytotoxic T cells. T-LGL, like PNH, can coexist with AA or MDS. T-LGL disease should be considered if increased LGLs are noted on the peripheral blood smear or if the patient has concomitant systemic autoimmune disease such as rheumatoid arthritis, which is known to be associated with T-LGL. Single lineage cytopenia is more common in T-LGL with clinical presentation of isolated neutropenia most typical or an anemia. Flow cytometry and testing for a clonal T-cell receptor gene rearrangement is appropriate when T-LGL is suspected. These patients also have a higher prevalence of *STAT3* mutations.

Another possible underlying cause of AA is FA, which can present with cytopenias in younger patients without other classic features of the disease. Therefore, diepoxybutane or mitomycin C testing to exclude chromosome fragility is important in patients with newly diagnosed AA <40 years of age, even in the absence of musculoskeletal abnormalities. FA is discussed further in Chapter 15.

The distinction between AA and hypoplastic MDS may be difficult to make, and increasing evidence suggests that immune-mediated mechanisms similar to those postulated to cause AA may contribute to the cytopenias associated with some cases of hypoplastic MDS and also normocellular or hypercellular MDS, even in the absence of a preceding diagnosis of AA. Such evidence includes the identification of clonal-activated cytotoxic T-cell populations in both AA and MDS, the coexistence of PNH and T-LGL clones in both AA and MDS, and improved blood counts in a subset of MDS patients treated with IST (see next section on myelodysplastic syndromes). Hypolobated neutrophils, dysplastic megakaryocytes, or abnormally localized and increased immature precursors favor a diagnosis of hypoplastic MDS rather than AA. Sometimes the only way to make the distinction between AA and MDS is by detection of an abnormal cytogenetic clonal population, but even this may not be diagnostic of MDS because some cytogenetically abnormal clones can be observed transiently in AA.

Table 18-3 Differential diagnosis of pancytopenia with a hypocellular bone marrow

Acquired aplastic anemia
Inherited aplastic anemia
Fanconi anemia
Dyskeratosis congenita
Shwachman-Diamond syndrome
Amegakaryocytic thrombocytopenia
Reticular dysgenesis
Hypoplastic myelodysplastic syndromes
Large granular lymphocytic leukemia (rare)
Hypoplastic paroxysmal nocturnal hemoglobinuria (PNH/aplastic anemia)

KEY POINTS

- AA is a diagnosis of exclusion and can result from intrinsic stem cell defects, immunologic impairment of hematopoiesis, or toxic effect of an exogenous exposure.
- PNH clones are frequently seen in patients with acquired AA.
- Chromosome abnormalities favor an MDS diagnosis over AA, but both karyotypic changes and clonal somatic mutations can sometimes be seen in patients with AA.
- Chromosome fragility tests to exclude FA and telomere length testing are important in children and younger adults <40 years of age presenting with idiopathic marrow failure.

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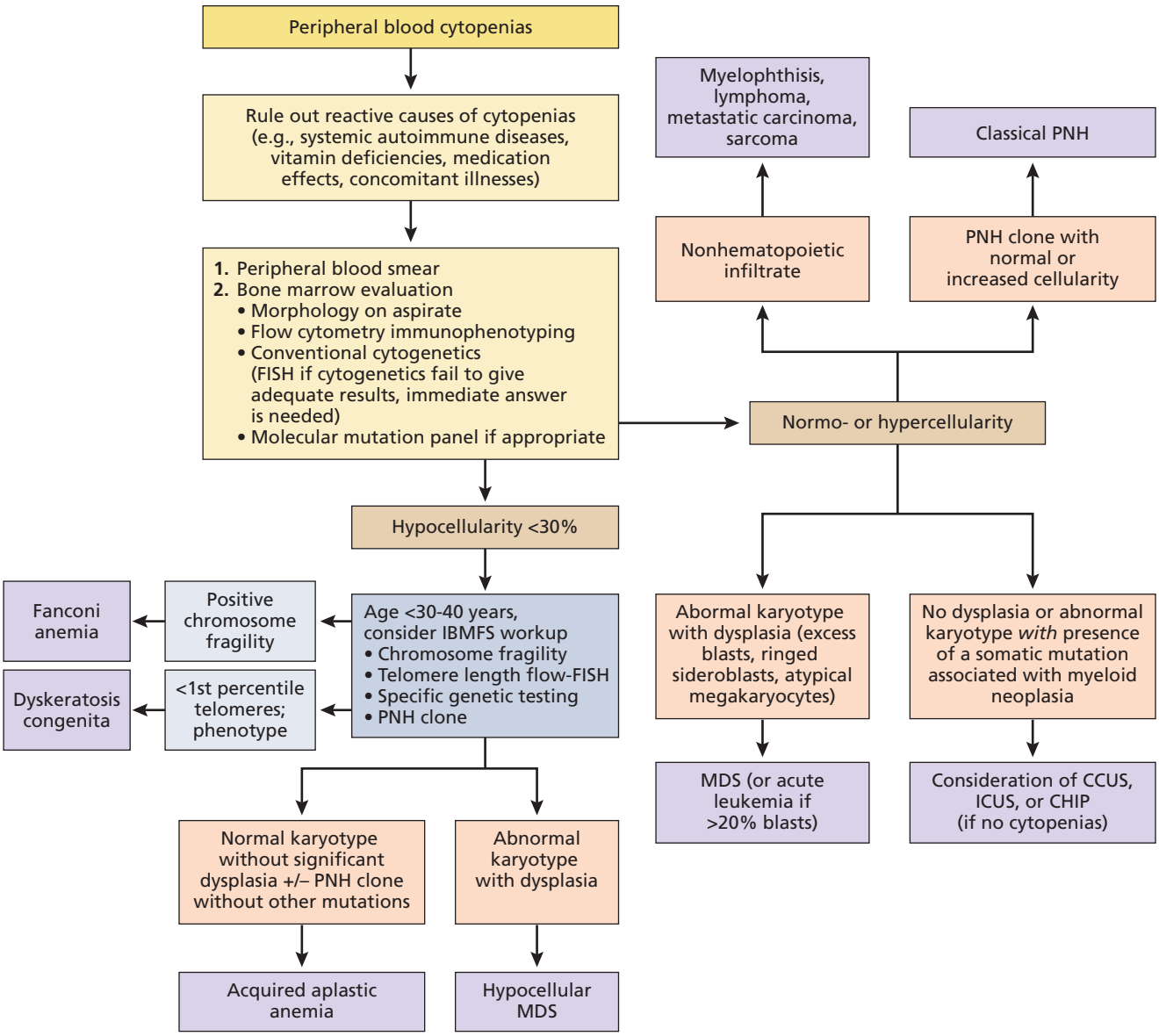
Peripheral blood cytopenias
Rule out reactive causes of cytopenias (e.g., systemic autoimmune diseases, vitamin deficiencies, medication effects, concomitant illnesses)
1. Peripheral blood smear
2. Bone marrow evaluation
• Morphology on aspirate
• Flow cytometry immunophenotyping
• Conventional cytogenetics (FISH if cytogenetics fail to give adequate results, immediate answer is needed)
• Molecular mutation panel if appropriate
Hypocellularity <30%
Age <30-40 years, consider IBMFS workup
• Chromosome fragility
• Telomere length flow-FISH
• Specific genetic testing
• PNH clone
Positive chromosome fragility
Fanconi anemia
<1st percentile telomeres; phenotype
Dyskeratosis congenita
Normal karyotype without significant dysplasia +/– PNH clone without other mutations
Abnormal karyotype with dysplasia
Acquired aplastic anemia
Hypocellular MDS
Normo- or hypercellularity
Myelophthisis, lymphoma, metastatic carcinoma, sarcoma
Nonhematopoietic infiltrate
Classical PNH
PNH clone with normal or increased cellularity
Abormal karyotype with dysplasia (excess blasts, ringed sideroblasts, atypical megakaryocytes)
MDS (or acute leukemia if >20% blasts)
No dysplasia or abnormal karyotype with presence of a somatic mutation associated with myeloid neoplasia
Consideration of CCUS, ICUS, or CHIP (if no cytopenias)

Figure 18-2 Diagnostic algorithm of primary marrow causes of pancytopenia. In patients with a hypocellular marrow, the main differential is between hypocellular MDS and AA. Normal cytogenetics and no significant dysplastic changes favor AA, whereas more pronounced dysplasia (micromegakaryocytes, left shift myelopoiesis with increase in blasts, significant dyserythropoiesis) and an abnormal cytogenetics favor MDS. Patients with AA and a PNH clone are classified as AA/PNH, which is distinct from classical PNH. It is important in appropriate patients to consider inherited causes of the marrow failure (IBMFS). In those with normal or increased marrow cellularity, differential includes a nonhematopoietic marrow infiltrating process (lymphomas, metastatic carcinoma, or sarcomas), MDS, and other primary marrow disorders (including AML if >20% blasts). More recently, as next-generation sequencing panels are sent for molecular mutations, the diagnoses of CHIP, CCUS, and ICUS are being made as well.

Therapy

Without treatment, almost all patients with SAA or very severe AA eventually die of infection or hemorrhagic complications. Therefore, such patients require urgent therapy once a diagnosis is confirmed. The decision to treat patients with AA is based on disease severity. Definitive treatment with either IST or allogeneic hematopoietic stem cell transplantation (HSCT) is necessary for patients with SAA (Figure 18-3). The standard of care for nonsevere AA is not established. Except for cases in which there is transfusion dependence, therapy is optional because survival is not affected by treatment. Rarely, patients with moderate AA can spontaneously recover normal hematopoiesis. Spontaneous remission is

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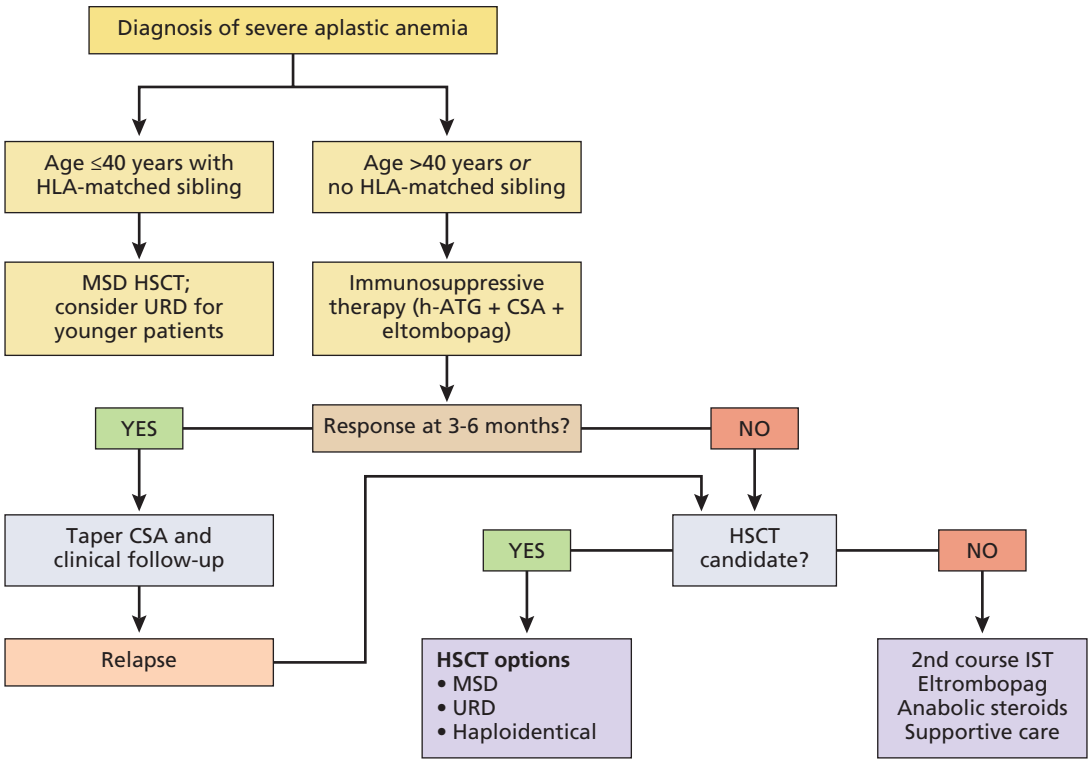

Figure 18-3 Algorithm for initial management of SAA. In patients who are not candidates for an upfront MSD HSCT, high-dose immunosuppression (likely with h-ATG plus CsA and eltrombopag [Epag]) should be the initial therapy. Response assessment occurs at 3 to 6 months, and the decisions on further intervention for nonresponders is based again on severity of disease. In patients who have persistent neutrophil count $<0.2 \times 10^9$/L, use of salvage therapies earlier is prudent. HSCT is favored to reconstitute hematopoiesis in appropriate patients. In those who are not suitable for transplantation and a repeat course of immunosuppression because of advanced age, comorbidities, lack of donor, poor performance, or personal preference, non-HSCT options can be considered earlier after refractoriness to initial course of therapy. URD, unrelated donor.

most often seen with drug-induced AA and usually occurs within 1 to 2 months of discontinuing the offending drug. Once the severity criteria are fulfilled, the type of treatment recommended is influenced by the patient's age and the availability of a matched sibling donor (MSD). A younger age (typically <40 years of age) and the presence of an MSD favor the use of allogeneic HSCT, while older age (>40 years old) and absence of an MSD favor the use of IST, which typically uses a combination of ATG, cyclosporine (CsA) or tacrolimus, and now with thrombopoietin receptor agonist eltrombopag (Epag). Attention to the timeline for reconstitution of hematopoiesis in these patients is critical for good outcomes.

Supportive care, transfusions, and hematopoietic growth factors

Supportive care is instituted to sustain blood counts (both hemoglobin and platelets) and alleviate symptoms and risks associated with pancytopenia. If transfusion is needed in a patient with AA, it is best to use irradiated, leukocyte-depleted blood products because of the risk for alloimmunization. Transfusions from related family members should be avoided because of the increased risk of subsequent graft rejection. If the patient is cytomegalovirus negative, it is best to use cytomegalovirus-negative blood products or leukocyte-depleted products. The role of preventive antibiotics in neutropenic patients is not well-defined.

Fungal and bacterial infections are a major cause of death in patients with SAA. However, an active fungal infection should not delay more definitive therapy, such as IST or HSCT. Vigilance as well as proactive use of prophylactic antibiotics (when deemed clinically appropriate), antivirals, and antifungals are recommended. Where possible, it is prudent to avoid agents associated with high rates of bone marrow suppression.

Granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor and erythropoiesis-stimulating agents (ESAs) have a limited role in AA. Most patients with AA have an elevated serum

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erythropoietin level and do not respond to recombinant erythropoietin. Although typical AA does not respond to myeloid growth factors either, some patients do improve neutrophil counts, and these growth factors may have a role in decreasing infectious morbidity while awaiting definitive treatment with IST or HSCT. In several randomized trials, the addition of granulocyte colony stimulating factor (G-CSF) to standard ATG and cyclosporine therapy did not improve the rates of hematologic response rate or survival. More recently, Epag was studied in combination with ATG/CsA; the results are discussed in the section on immunosuppressive therapy.

Corticosteroids are ineffective, increase the risk of infection, and should not be used as therapy in AA. The role of corticosteroids in SAA is limited to serum sickness prophylaxis with concurrent ATG administration. Androgens may have a supportive role in some patients throughout the treatment course of AA. Androgens, however, should not be used as primary upfront therapy.

KEY POINTS

- If transfusions are needed in a patient with AA, use irradiated, leukocyte-depleted blood products not from family members (especially in transplant candidates).
- Transfusions should be used judiciously in symptomatic anemic individuals or in those at higher risk for bleeding.
- AA does not usually respond to G-CSF or erythropoietin, but responses have been noted with Epag as a single agent and in combination with ATG/CsA.
- Corticosteroids should not be used as therapy in AA except as prevention of serum sickness in patients receiving ATG.

Hematopoietic stem cell transplantation

Adolescents and young adults (age <40 years) meeting the criteria for severe disease who have an HLA-MSD should proceed directly to HSCT, as this is potentially curative. An advantage of hematopoietic stem cell transplantation over standard IST is a marked reduction in the risk of relapse and abrogation of the risk for the development of clonal disorders such as MDS and PNH. Despite this, the risks of acute and chronic graft-versus-host disease (GVHD) remain a challenge after HSCT when donors other than matched siblings are used.

In AA, the pretransplantation conditioning regimen primarily is administered to provide immunosuppression, which enables the donor stem cells to engraft and also eliminate activated immune cells that may be causing the marrow aplasia. Cyclophosphamide (50 mg/kg/d × 4 days) with or without ATG is commonly used for conditioning before stem cell transplantation. Although this regimen is nonmyeloablative, the immunosuppression is sufficient to allow engraftment in most cases. Avoidance of total body irradiation and busulfan reduces transplant-related complications such as mucositis, GVHD, second malignancies, and infertility. Alternative regimens using fludarabine, cyclophosphamide, and antithymocyte globulin are increasingly being used. Survival rates following matched sibling allogeneic bone marrow transplantation (BMT) have steadily improved since the 1970s largely because of improved supportive care, improved typing, and better GVHD prophylaxis. Bone marrow has been a traditional source for the stem cell graft, but the use of peripheral blood stem cells has gained in popularity in the past 10 to 15 years. This practice has resulted in an untoward consequence in transplanted AA patients, where several reports in the recent years from Europe and the United States show an increase rate of GVHD with stem cells derived from mobilized peripheral blood when compared with a bone marrow source. In contrast to allogeneic HSCT undertaken for malignant disorders, where GVHD offers potential graft-versus-tumor benefits, GVHD is to be avoided at all costs in the AA setting, because its occurrence is associated with decreased survival and long-term quality of life. Thus, bone marrow is the preferred source of HSCs in AA patients undergoing HSCT.

Late BMT-related complications such as chronic GVHD occur in up to one third of patients, with many of these patients requiring long-term therapy for their GVHD. Standard prophylactic therapy for GVHD includes a calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate or posttransplant cyclophosphamide. Patient age and the type of allograft (HLA-matched sibling, unrelated, or mismatched donors) are the most important factors influencing outcome. In patients under 30 years old, the cure rate after HLA-matched sibling BMT ranges from 70% to 90%. However, the risk of GVHD steadily increases with age, leading to reduced survival. A recent Cochrane review concluded that no firm conclusions can be drawn about the comparative effectiveness of first-line allogeneic HSCT of HLA-matched sibling donors and first-line IST of patients with acquired SAA.

For older patients, reduced-intensity transplantation conditioning regimens using low doses of total body irradiation or fludarabine have shown promise in reducing rejection rates. In recent years, however, outcomes with matched-unrelated-donor HSCT have improved likely because of more stringent donor selection with high-resolution-molecular tissue typing, less toxic and more effective conditioning regimens, and higher quality transfusion and antimicrobial supportive care. In some reports

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in children, outcomes with a matched-unrelated HSCT have compared favorably to those observed with sibling donors, and this treatment modality is becoming the preferred salvage treatment modality in younger patients who fail an initial course of immunosuppression when a matched-unrelated histocompatible donor is available.

Outcomes with mismatched-unrelated umbilical cord donors are not as favorable, with higher rates of graft rejection, infectious complications, acute and chronic GVHD, and transplant-related mortality. Newer results in haploidentical HSCT have increasing success with lower toxicity than previously reported. These alternative donor transplants usually are undertaken at the time that refractory or relapsed disease is diagnosed (Figure 18-3). There are ongoing investigations into use of alternative donors earlier in a patient's course.

KEY POINTS

- Outcomes with HSCT are better in younger patients (especially patients <20 years old); in patients >40 years old, transplantation-related mortality and morbidity may be increased somewhat.
- Bone marrow is the preferred source of stem cells in AA, not peripheral blood stem cells, unlike the situation with hematological neoplasms.
- Alternative transplantation should be reserved for patients for whom an initial course of immunosuppression has failed.

Immunosuppressive therapy

The principal immunosuppressive agent used in SAA is ATG, which is manufactured by delivering human T cells to a horse or rabbit. The immunized animal then produces antibodies against antigens expressed on the surface of a T cell, which subsequently are harvested and purified. The resulting polyclonal animal serum has lymphocytotoxic properties, and administration to humans leads to varying degrees of lymphocyte depletion. Several in vitro and in vivo differences are observed between the 2 types of ATG despite a similar manufacturing process. Rabbit ATG (r-ATG) has a longer half-life and results in a more durable lymphocyte depletion compared to horse ATG (h-ATG). A difference in T-cell binding affinity, cytokine release, and T-cell subset depletion and reconstitution has also been shown to be distinct between the ATGs.

Initial investigations using h-ATG or CsA alone in AA were succeeded by studies of h-ATG and CsA in combination, with improved response rates over monotherapy, becoming the standard regimen. Multiple efforts to add further immunosuppression to improve outcomes beyond h-ATG/CsA have been disappointing. Addition of mycophenolate mofetil, G-CSF, or sirolimus did not improve hematologic responses or decrease the relapse and clonal evolution rates. The use of more lymphocytotoxic agents such as r-ATG, alemtuzumab, or cyclophosphamide led to worse outcomes than with h-ATG/CsA in randomized studies, resulting from a lower response rate and/or excess toxicities.

The usual time to response to standard treatment with h-ATG/CsA therapy in SAA is approximately 10 to 12 weeks. In most studies, responses are defined as achieving blood counts that no longer fulfill criteria for severe disease, as well as transfusion independence. Total restoration of blood counts occurs in a minority of patients, and recovery can be protracted.

The overall response rate at 3 months in patients receiving h-ATG/CsA is between 60% and 80%. Hepatitis-associated and drug-related AA appears to be as equally responsive to IST as idiopathic AA. Although most patients who respond to IST do so by 6 months, in a small minority of patients, time to recovery may be longer. Achieving hematologic response (partial or complete) to immunosuppression is very important in SAA because it strongly associates with long-term survival.

Recently Epag was studied in a larger, 92-patient prospective trial at the National Institutes of Health in combination with ATG/CsA. This trial demonstrated higher rates of response compared to historical controls (80% to 94% compared to 66%), and response was associated with a longer duration of Epag exposure (up to 6 months). The addition of Epag was well-tolerated, with only rash as a severe adverse event in 2 patients. With early tapering of the CsA in the initial phases of the study, there was a relapse rate of 32%, resulting in an amendment to continue the CsA for 2 years. Concerns of increased clonal evolution have been postulated with clonal cytogenetic evolution in 7 patients at 2 years, but this has not been shown to be more than previous reports, and longer-term follow-up is ongoing. The addition of Epag is approved for use with IST as standard of care for initial treatment of SAA.

Both horse- and rabbit-derived ATGs have activity in SAA, with most of the experience with horse occurring in the upfront setting and experience with rabbit in the salvage setting. A repeat course of r-ATG and CsA may be given to h-ATG–refractory patients, which results in additional responses in approximately 35% of patients. In responders to h-ATG/CsA, relapse has been reported in 35% of patients by 5 years. Relapses can be related temporally to the discontinuation of CsA or to the reduction of its dose. Cyclosporine should be continued for at least

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6 months. The benefit of a taper in abrogating or reducing relapse rates has not been confirmed in prospective studies; however, most practicing hematologists institute a slow CsA taper after 6 months in an attempt to prevent hematologic relapses. Relapsed patients may respond to an increased dose or reintroduction of CsA or a second course of r-ATG, which results in hematologic responses in about 60% to 70% of patients. Approximately 25% of patients remain chronically dependent on CsA to maintain adequate blood counts. Aggressive taper is usually not beneficial to the patient, whereas active titration to avoid side effects (especially nephrotoxicity) is prudent.

The greater lymphocytotoxicity of r-ATG and its effectiveness in salvaging refractory and relapsed SAA patients prompted its use as initial therapy with the anticipation that it would be superior to h-ATG. In a randomized study, however, results with r-ATG were disappointing. The hematologic response rate with r-ATG was 37% compared with 68% for h-ATG at 6 months, and survival was inferior in the r-ATG arm. These results suggested that h-ATG/CsA remains the preferred first-line IST in SAA. As alternative therapy to h-ATG/CsA, high-dose cyclophosphamide has been used, with response rates comparable to that of h-ATG/CsA but with early reports suggesting fewer rates of relapse and clonal evolution.

Eltrombopag has demonstrated a role in refractory AA, as a small trial showed improvements in blood counts in patients with SAA who were refractory to at least 1 course of immunosuppression. A hematologic response rate of approximately 40% has been reported with this single agent, with multilineage responses observed. This outpatient oral therapy was well-tolerated and is approved for use after insufficient response to initial IST with ATG and CsA alone.

Long-term follow-up and prognosis

Clonal outgrowth with secondary hematological malignancies and impaired fertility are among the most worrisome late effects of IST and HSCT. Aecent follow-up study from Tichelli et al. with a median follow-up of 11.7 years of newly diagnosed SAA patients who received ATG/CsA with or without G-CSF found no difference in long-term effects of G-CSF on response to therapy, overall survival, event-free survival, clonal evolution, need for HSCT, and secondary complications. Patients treated with IST have a 1% to 5% chance of secondary hematological malignancies with clonal evolution to MDS or clinical PNH. Routine annual monitoring should be performed.

Although 40% to 50% of patients with AA have PNH clones at presentation, most are small and evolution to frank PNH is relatively infrequent. PNH that occurs after treatment, however, is frequently subclinical and is rarely associated with overt hemolysis or thrombosis. More concerning is evolution to MDS, which is most frequently associated with either monosomy 7 or a trisomy 8 karyotype. Evolution to MDS can occur in up to 10% to 20% of patients in the first 20 years after diagnosis, an event usually associated with a decrease in blood counts or refractoriness to immunosuppression. The prognosis of patients with chromosome 7 abnormalities is generally poor, whereas those with trisomy 8 can respond to IST.

Other cytogenetic abnormalities can be identified in follow-up of AA, which may not necessarily signify progression to MDS. Some of these abnormalities may be transient and may not be associated with dysplastic marrow findings, worsening in blood counts, or refractoriness to further therapies. The exception is the appearance of monosomy 7, which commonly is associated with frank dysplasia, with the only curative approach being an HSCT from a related or alternative donor.

Molecular testing is an evolving area of active research in the AA field and is primarily still performed on a research rather than a clinical basis. As many as 60% to 70% of acquired AA patients demonstrate clonality at the time of diagnosis, using sensitive next-generation sequencing and array-based karyotyping (comparative genomic hybridization) modalities. Unfortunately, these clones are often not eliminated posttherapy and are frequently the source of relapse and/or progression. Recurrent genetic abnormalities in *ASXL1*, *DNMT3A*, *TET2*, *PIGA*, and *BCOR* genes have been described in AA. Certain mutations (*BCOR*, *BCORL1*, and *PIGA)* were found to have a better response to IST and higher rate of overall and progression free survival; whereas *DNMT3A* and *ASXL1* were associated with worse outcomes. However, clones bearing these markers maybe variable in predicting response to therapy, survival, or may disappear with time. Discussion of clonal hematopoiesis is noted in the "Clonal hematopoiesis" section and depicted in Figure 18-1B.

Rarely, AA may develop in pregnancy. Spontaneous remission can occur in 25% to 30% of patients, often upon birth or termination of the pregnancy. CsA may be a safe drug antenatally in such patients. Complications appear to be more likely in pregnant patients with low platelet counts and associated PNH.

Overall survival is approximately 70% in patients over 16 years of age. HSCT using an MSD is indicated as a frontline approach in children and patients up to 20 years. However, approximately 70% of patients do not have an MSD. Further, clonal evolution often occurs in patients with AA and these patients do less well long-term.

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KEY POINTS

- Allogeneic stem cell transplantation from a matched sibling donor is the treatment of choice for patients with SAA in children and young adults.
- For older patients, those without sibling donors, and those who refuse transplantation or have significant comorbidities that preclude HSCT, immunosuppression with h-ATG, cyclosporine, plus Epag combination should be initiated as soon as possible once the diagnostic workup is completed.
- Outcomes with alternative donors including matched-unrelated-donor and haploidentical transplantation have improved in the recent years. Now these options may be considered in appropriate treatment-naïve patients as well as the preferred salvage treatment in those who fail an initial course of immunosuppression.
- Relapses occur in about 1/3 of responders to h-ATG plus cyclosporine, but often respond well to reinstitution of IST.
- Repeat courses of r-ATG and cyclosporine may be given to refractory patients if truly ineligible for HSCT, resulting in a salvage rate of approximately 35%.
- Eltrombopag is approved for therapeutic use in SAA patients who have an insufficient response to initial IST and is an option for those who are not eligible for HSCT because of lack of a histocompatible donor, age comorbidities, or personal preference.
- Clonal evolution to MDS can occur in 10% to 20% of patients post-IST.

Paroxysmal nocturnal hemoglobinuria

In acquired AA, PNH clones can be detected by flow cytometry in 40% to 50% of cases, but these are usually small (<10% of cells). A PNH clone can expand later in the course of disease leading to frank hemolysis; this occurs most commonly in patients with larger preexisting PNH clones at diagnosis. PNH clones can remain stable over time or reduce in size, having no clinical consequence. Indicators of the presence of a PNH clone include elevated lactate dehydrogenase (LDH), absent haptoglobin, increased reticulocytes, and erythroid predominance in the marrow.

Definition

PNH is a rare clonal HSC disorder that manifests with a chronic intravascular hemolytic anemia from uncontrolled complement activation, a propensity for thrombosis, and BMF. The hemolysis is largely mediated by the alternative pathway of complement. These clinical manifestations result from the lack of specific cell surface proteins, CD55 and CD59, on PNH cells resulting from a somatic mutation in the *PIGA* gene in HSCs, which results in failure to synthesize the glycosylphosphatidylinositol (GPI) anchor.

Pathophysiology

Hemolysis in PNH is complement mediated and is a direct result of the mutated PNH cells acquiring a deficiency of complement regulatory proteins. In PNH, because of the defect of the enzyme encoded by the mutant *PIGA* gene (Figure 18-4A), the first step in biosynthesis of the GPI anchor protein (AP) cannot be completed normally (Figure 18-4B), and all GPI-anchored proteins are absent on the surface of progeny cells of all hematopoietic lineages derived from the affected stem cell with increased susceptibility to hemolysis (Figure 18-4C and 18-4D).

The intravascular hemolysis in PNH is caused by the lack of GPI-anchored proteins (CD55 and CD59) that attenuate complement activation on the surface of erythrocytes. Depending on the type of mutation in the *PIGA* gene, various degrees of CD55 and CD59 deficiency can occur. Patients with PNH may have in their circulation an admixture of normal complement-resistant red blood cells (so-called PNH I cells), as well as mildly (PNH II) or markedly (PNH III) abnormal complement-sensitive cells. The difference in the proportion of these red blood cell (RBC) populations contributes to the variability in intravascular hemolysis seen in patients.

PNH patients have a propensity for thrombosis. Several theories have been postulated to account for this hypercoagulability, but the mechanism has not been clearly defined. It is believed that thrombophilia in PNH is related to the degree of hemolysis and thereby indirectly related to the size of PNH clone. Possible prothrombotic pathways include platelet activation by complement components, procoagulable microparticles derived from GPI-deficient erythrocytes, or slowing of the microcirculation because of vasoconstriction induced by products of hemolysis. It also has been suggested that intravascular hemolysis exposes red blood cell phospholipids that may serve to initiate coagulation.

PNH is also a disorder of marrow failure. PNH clones expand only in the context of immune-mediated BMF, explaining the close association between AA and PNH. According to the most predominant hypothesis, PNH stem cells, which can be found in very low frequencies in healthy individuals, have a selective advantage in certain circumstances of immune dysregulation. Under conditions of T-cell–mediated immune attack on HSCs, GPI-deficient stem cells appear to thrive because of selective survival advantage compared with healthy stem cells, which facilitate their expansion. This close association between immune-mediated depletion of normal stem and progenitor cells explains the coexistence of hematopoietic failure and frequent cytopenias related to impaired blood cell production (Figure 18-5).

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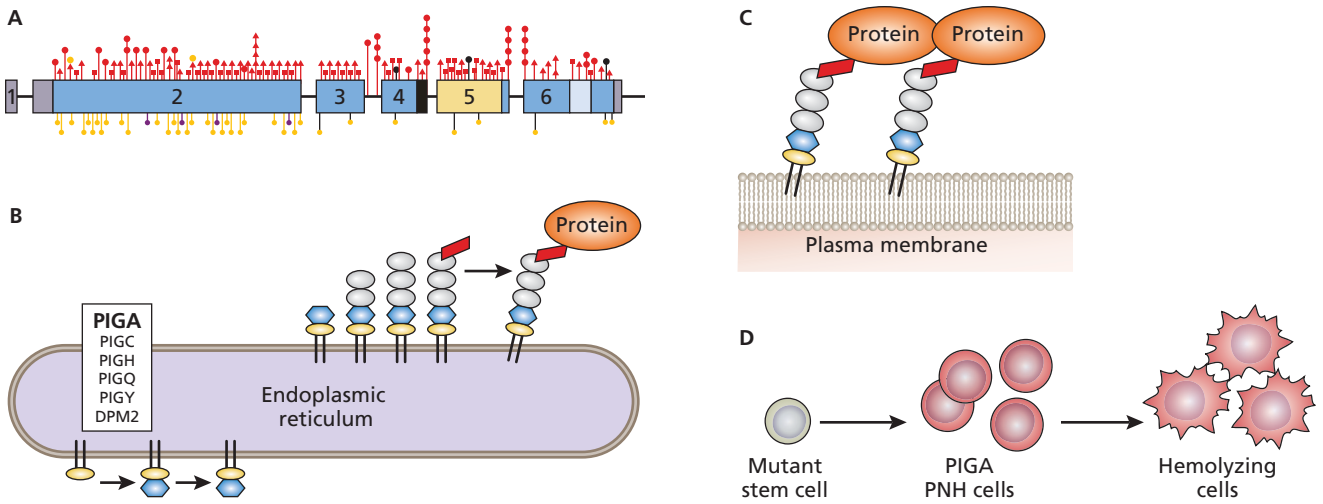

Figure 18-4 Pathogenesis of PNH. (A) In hematopoietic stem cells, acquired somatic mutations of the *PIGA* gene may occur. This controls the key step in the biosynthesis of GPI anchor proteins. (B) GPI anchor biosynthesis takes place in the endoplasmic reticulum. *PIGA* is 1 of 7 subunits involved in the first step of GPI anchor biosynthesis. (C) After multiple steps, including protein attachment to the GPI anchor and fatty acid remodeling, the GPI-anchored protein should be transported to the plasma membrane. This cannot occur in PNH patients. (D) These mutations (in panel a) can decrease the function or totally inactivate the enzyme encoded by *PIGA*. As a consequence, all proteins using this type of anchor are deficient from the membrane of affected progeny derived from the mutant stem cells and cause the PNH phenotype and hemolysis.

Laboratory findings and diagnosis

The diagnosis of PNH is both a laboratory and a clinical diagnosis, which can show numerous and varied presentations. It may present with a Coombs-negative hemolytic anemia, pancytopenia, abdominal pain, renal impairment, hemoglobinuria, and/or thrombosis. PNH can arise de novo or evolve from acquired AA. Currently, the diagnosis of PNH is secured by abnormal laboratory measures including a reticulocyte count, lactate dehydrogenase levels, complete blood count indicative of hemolysis, and peripheral blood flow cytometry to detect the deficiency of the GPI-AP. This absence of GPI-APs is detected after staining cells with monoclonal antibodies (eg, CD55, CD59) and/or a reagent known as fluorescein-tagged proaerolysin variant that binds a portion of the GPI anchor.

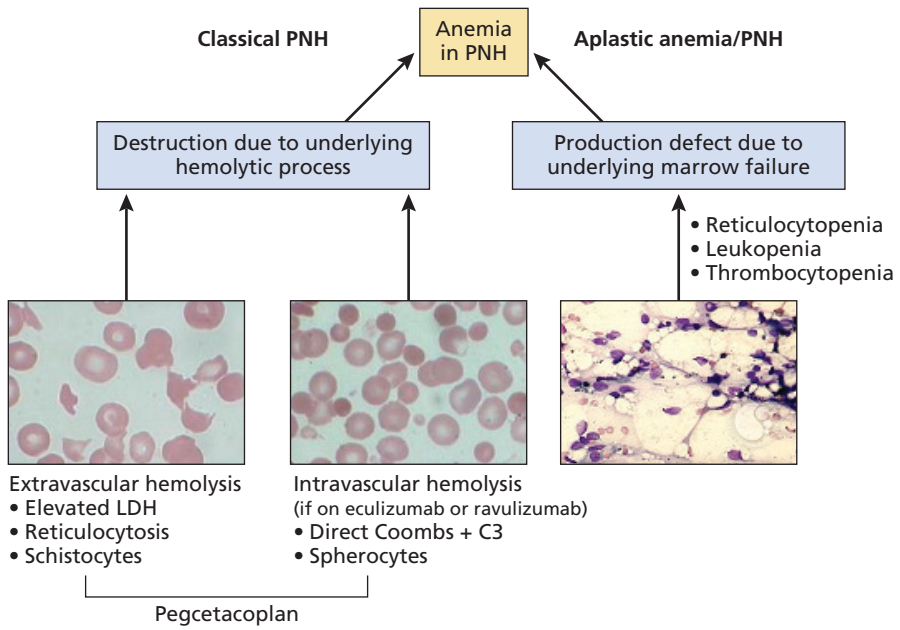

Figure 18-5 Mechanisms of anemia in PNH. Anemia in PNH can be a result of increased RBC destruction because of intravascular hemolysis of glycosylphosphatidylinositol-deficient RBCs, decreased production of RBCs because of immune-mediated bone marrow failure, or a combination of these 2 mechanisms. Hemolysis can be compensated for by increased production (patients with increased reticulocytes) or compensation may be inadequate (patients with low reticulocyte counts).

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The erythrocytes may be classified as type I, II, or III PNH cells, as noted previously. It should be noted that testing of a PNH clone solely in erythrocytes is not adequate for evaluation of PNH, because hemolysis and transfusions may greatly underestimate the size of the clone. For these reasons, granulocyte and monocyte clones are frequently detected when erythrocyte clones are not. In patients with brisk hemolysis associated with PNH, macrocytic anemia caused by compensatory reticulocytosis is typically present (if hematopoiesis is not suppressed), but some PNH patients with iron deficiency because of chronic urinary iron losses may have microcytic red blood cell indices. Elevated LDH and absent haptoglobin together with urine hemosiderin indicate the presence of intravascular hemolysis. Various degrees of thrombocytopenia and neutropenia also may be present in patients with PNH associated with AA. In the absence of AA, the bone marrow shows relative expansion of erythroid series, and most often is hypercellular.

There is no universally accepted classification scheme. Recently the PNH International Registry classified PNH into the following 3 categories: (1) hemolytic or classical PNH, (2) AA-PNH, and (3) intermediate PNH. Patients with hemolytic PNH tend to have near-normal neutrophil and platelet counts, an LDH >2 times the upper limit of normal, a normocellular bone marrow, an elevated reticulocyte count, and a relatively large population of PNH granulocytes (usually >50%). AA-PNH patients are more pancytopenic and tend to have a hypocellular bone marrow, a relatively low reticulocyte count, and a smaller percentage of PNH granulocytes. It is also important to recognize that these categories have limitations and a patient's classification can change over time. For example, patients with AA-PNH may experience improved hematopoiesis associated with expansion of their PNH clone and later meet criteria for hemolytic PNH. Less commonly, patients with hemolytic PNH may develop AA-PNH.

KEY POINTS

- PNH is an acquired clonal HSC disorder characterized by deficiency of GPI-linked proteins in blood and bone marrow cells because of a somatic mutation in the *PIGA* gene.
- Patients with PNH experience chronic hemolytic anemia (intravascular) from uncontrolled complement activation. They may also suffer from a propensity for thrombosis and BMF.
- Flow cytometric techniques to identify cell populations lacking GPI-linked proteins, such as CD55 and CD59, confirm the diagnosis of PNH and are used to estimate the size of PNH clone.

Clinical manifestations

Chronic hemolytic anemia of various degrees is the most common manifestation of PNH. Despite the name of the disease, hemoglobinuria with darker-stained urine at a particular time of the day is reported by only a minority of patients. Symptoms related to hemolysis include back and abdominal pain; headache; smooth muscle dystonias, such as esophageal spasm and erectile dysfunction (caused by scavenging of nitric oxide by free plasma hemoglobin), and severe fatigue irrespective to the degree of anemia. Exacerbations of hemolysis can occur with infections, surgery, or transfusions. If severe, hemolysis can result in acute renal failure because of pigment nephropathy. Icterus often is present intermittently and typically worsens during hemolytic exacerbations.

The most concerning complication of PNH is thrombosis. It is the leading cause of death in the disease. Thrombosis may occur at any site in PNH, venous or arterial. Common sites include intra-abdominal (hepatic, portal, splenic, or mesenteric) and cerebral (cavernous or sagittal sinus) veins, with hepatic vein thrombosis (also known as Budd-Chiari syndrome) being the most common. Deep venous thrombosis, pulmonary emboli, and dermal thrombosis are also prevalent. For unclear reasons, thrombotic complications are less common in PNH patients of Asian descent. The thrombotic propensity is particularly enhanced during pregnancy. Clinically, the complication of thrombosis is more prevalent in patients as the PNH clone increases in size. Thrombosis may occur in any PNH patient, but those with a large percentage of PNH cells (>50% granulocytes) are at greatest risk. Complement inhibition with eculizumab, ravulizumab, or pegcetacoplan are the most effective means to stop thrombosis in PNH.

Patients with PNH suffer from anemia but may also have other cytopenias depending on the degree of the associated marrow failure. The marrow failure component of PNH can vary from subclinical disease to SAA and may be categorized as an overlap syndrome of AA/PNH. The PNH clone is often considered a marker of an immune form of marrow failure, as it may predict response to IST in AA; therapies directed at PNH hemolysis do not improve the patient's component of underlying marrow failure.

Treatment

The variability in the clinical manifestations of PNH makes it necessary to individualize the treatment plan. Anemia is often the dominant issue to be addressed. Anemia resulting from hemolysis should be distinguished from BMF-related anemia. Chronic hemolysis should be treated with supportive measures, such as transfusions,

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supplementation of folate and iron, and, in the context of renal failure, recombinant erythropoietin administration. Table 18-4 outlines standards for clinical care for these patients.

Two humanized monoclonal antibodies, eculizumab and ravulizumab, (C5 inhibitor) to the C5 terminal complement protein and 1 pegylated peptide, pegcetacoplan, (C3 inhibitor) targeting the C3 protein are United States Food and Drug Administration (FDA)-approved for use in patients with PNH. The C5 inhibitors have shown efficacy in decreasing intravascular hemolysis, decreasing the need for transfusions, the risk of thrombosis, and improving the quality of life in patients with PNH. Similarly, pegcetacoplan has shown improvement with targeting intra- and extravascular hemolysis. Treatment is associated with few complications, but because the terminal components of complement are important to protect from *Neisseria meningitidis*, vaccination against this microorganism is important before initiation of therapy (at least 2 weeks in advance). The decision about when to start therapy needs to take into consideration the degree of chronic hemolysis, frequency of acute hemolytic attacks, severity of constitutional symptoms, thrombotic history, and frequency of transfusions—parameters that should be balanced against the need for chronic lifelong infusions and the high cost of the drug.

Table 18-4 Clinical care of patients with PNH

Diagnosis
PNH by FLAER assay
LDH
Reticulocyte count
Complete blood count
Therapy
Eculizumab IV
Loading: 600 mg weekly × 4 weeks
Maintenance (followed 1 week later): 900 mg every 2 weeks thereafter
Modification to frequency or dose can be considered if ongoing hemolysis
Ravulizumab IV
Dose is weight based with loading dose followed by maintenance dose once every 8 weeks starting 2 weeks after the loading dose
Pegcetacoplan subcutaneous injection
Set dose of 1080 mg twice weekly; dose modification can be considered
Consideration of HSCT in suboptimal responders
Monitoring while on therapy
At least monthly
LDH, reticulocyte count, complete blood count, chemistries
At least yearly
PNH by FLAER assay
If concern for extravascular hemolysis
Direct antiglobulin test

FLAER, fluorescein-tagged proaerolysin variant; IV, intravenous.

If a diagnosis of a thrombosis is made in a PNH patient, aggressive treatment is warranted. Anticoagulation and therapy with a C5 or C3 inhibitor are indicated for acute thrombotic events; however, primary prophylactic anticoagulation has not been well established to be beneficial in PNH. Anticoagulation after the acute event in a PNH patient well maintained on therapy may not be necessarily lifelong.

The majority of classical PNH patients respond to a C5 or C3 inhibitor; however, the hemoglobin response is highly variable and may depend on underlying BMF, concurrent inflammatory conditions, genetic factors, and the size of the PNH red cell clone following therapy. Patients do require close monitoring while on treatment. Unfortunately, not all patients have their disease-specific needs met by a C5 or C3 inhibitor because it does not improve underlying BMF. There are also reports of patients who have a coexistent autoimmune disease with ongoing activation of complement from their underlying disease, which leads to suboptimal responses from treatment. Transient breakthrough intravascular hemolysis can be observed following viral or bacterial infections. Pregnancy is a hypercoagulable state itself, and there have been concerns both about the potential for increased maternal and fetal morbidity in a pregnant patient as well as the safety of eculizumab therapy in pregnancy. There are multiple case reports and series reporting successful pregnancies in patients on eculizumab only. However, what has been observed is the tendency for breakthrough hemolysis at later stages of pregnancy that requires reduced dosing interval by the third trimester. Japanese patients can be another group of suboptimal responders to C5 inhibitors. They may carry a single missense C5 heterozygous mutation, c.2654G→A, which prevents binding and blockade by C5 inhibition while retaining the functional capacity to cause hemolysis. The polymorphism accounts for the poor response to therapy in patients carrying the mutation. Lastly, C5 inhibitors only compensates for the CD59 deficiency on PNH erythrocytes, but not the CD55 deficiency. Thus, PNH patients on therapy may accumulate C3 fragments on their CD55-deficient red cells, leading to extravascular hemolysis through the accumulation of opsonins that are recognized by the reticuloendothelial

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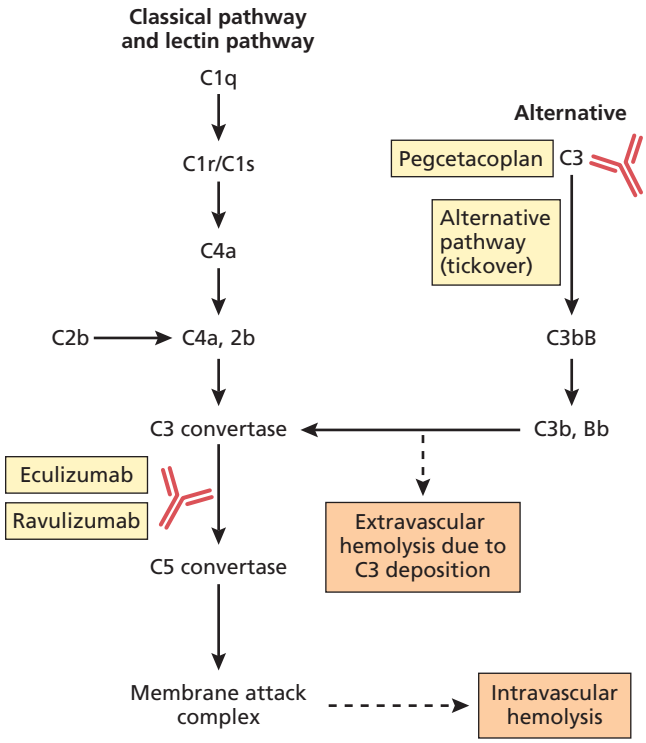

Figure 18-6 The complement cascade, paroxysmal nocturnal hemoglobinuria, and complement inhibitors. PNH cells have a deficiency in glycosylphosphatidylinositol-anchored proteins on their cell surface. Absence of CD55 and CD59 leads to uncontrolled complement activation on the surface of PNH cells. Deficiency of CD59 increases Membrane attack complex (MAC) formation and induces intravascular hemolysis, which is central to the pathophysiology of PNH. Deficiency of CD55 leads to increased C3 convertase activity and C3d-associated extravascular hemolysis. Eculizumab and ravulizumab are humanized monoclonal antibodies that targets C5 for the treatment of PNH. By preventing C5 activation, eculizumab and ravulizumab prevent the formation of the MAC, leading to a significant reduction in intravascular hemolysis of PNH cells. Use of C5 inhibitors can lead to increased extravascular hemolysis in some patients. Pegcetacoplan is a pegylated peptide that targets C3 and controls intra- and extravascular hemolysis for the treatment of PNH.

system (Figure 18-6). Laboratory evidence of extravascular hemolysis includes increased reticulocytes, persistent anemia, and often direct antiglobulin testing that is positive for C3 deposition. These patients may remain asymptomatic, but others have symptomatic anemia and remain dependent on transfusions. Thus, the recent approval of pegcetacoplan which targets proximal complement inhibition reduces C3 accumulation on PNH erythrocytes to address the shortcomings of C5 inhibitors in PNH. Note that oral therapies targeting with proximal complement system, factor B (iptacopan) and D (danicopan) inhibitors are currently in clinical trials as monotherapy and/or in combination with a C5 inhibitor

Life-threatening and fatal meningococcal infections have occurred in patients treated with C5 or C3 inhibitors because of the complement blockade and inability to fight encapsulated pathogens. The risk of these infections is low when patients are properly vaccinated with the meningococcal vaccination at least 2 weeks prior to administering the first dose of therapy. In patients for whom the risks of delaying therapy outweigh the risk of developing a meningococcal infection, a fluoroquinolone (ciprofloxacin) can be given as a bridge. Furthermore, any infection can increase complement and increase hemolysis, even in well-managed patients with stable therapy. Instructions to notify providers of fevers, headaches, or other symptoms should be provided to all patients so that prompt medical attention is available.

The approach to severe BMF associated with PNH should be similar to that taken for SAA. IST with h-ATG and cyclosporine can be effective in improving blood counts and may allow for better compensation of hemolysis. Immunosuppressive drugs, however, are mostly ineffective in patients with purely hemolytic forms of PNH who have adequate marrow reserve.

HSCT is the only curative therapy for PNH. However, it is not recommended as upfront therapy in the complement inhibitor era given the risks of transplant-related morbidity and mortality. HSCT is a reasonable therapeutic option in patients who do not respond to therapy or those patients who have severe pancytopenia caused by underlying BMF. The transplant paradigm pursued is often with reduced-intensity conditioning regimens, as myeloablation is not required to eradicate the PNH clone.

Prognosis

Patients diagnosed with classical PNH without leukopenia, thrombocytopenia, or other complications maintained on complement inhibitor therapy can anticipate long-term survival and improved outcomes. Thrombotic events, progression of the marrow failure component, clonal evolution to MDS/acute myeloid leukemia (AML), and age >55 years at diagnosis have been correlated with a poorer prognosis for PNH patients.

KEY POINTS

- Eculizumab and ravulizumab are monoclonal antibodies against the C5 terminal complement component; both compensate to block intravascular hemolysis in patients with symptomatic PNH, reduce thrombotic events, and alleviate the need for transfusions in most cases.

Key Points continue

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KEY POINTS *(continued)*

- Pegcetacoplan is a pegylated peptide targeting the C3 proximal complement component, blocking both intra- and extravascular hemolysis, improving anemia.
- Prompt evaluation of PNH patients is indicated when symptoms are suggestive of thrombosis because the risk of clotting is high.
- Treatment of bone marrow aplasia with IST will not eliminate the PNH clone and is generally ineffective in primary hemolytic PNH. Immunosuppression, however, may be helpful in patients with AA/PNH syndrome.
- Allogeneic HSCT has curative potential but is indicated only in patients with severe cytopenias and severe thrombotic complications refractory to medical therapy.

Myelodysplastic syndromes

Introduction

MDS include a heterogeneous group of clonal, acquired disorders characterized by ineffective hematopoiesis, resulting in peripheral blood cytopenias. MDS carries a variable risk of progression to AML. AML is defined by the World Health Organization (WHO) as >20% blast cells in the marrow or blood, or the presence of certain AML-defining karyotypes such as t(15;17); thus, all patients with MDS have <20% marrow blasts, by definition.

MDS may arise de novo—80% to 85% of cases are idiopathic—or may be secondary to a recognized exposure to a DNA-damaging agent. Secondary or therapy-related MDS (t-MDS) can be induced by drugs that alkylate DNA bases, inhibitors of topoisomerase II, therapeutic or accidental exposure to ionizing radiation, or environmental or occupational exposure to other DNA toxins, such as hydrocarbons. Proving a causal connection between a suspected exposure and subsequent development of MDS can be challenging, but the presence of a relevant history with a complex karyotype (defined as at least 3 acquired chromosome abnormalities), abnormalities of chromosomes 5 and 7, or somatic *TP53* mutation is suggestive of t-MDS.

Among the potential peripheral blood cytopenias, anemia (often macrocytic) is the most commonly observed cytopenia in MDS, present in >90% of cases at diagnosis. Dysplastic cell morphology (discussed in the "Diagnostic evaluation" section) is diagnostically important, reflects failure of cells to differentiate and mature normally, and often is accompanied by cellular dysfunction that exacerbates the signs or symptoms of cytopenias. As a result, the infection and bleeding risks in MDS correlate poorly with the circulating neutrophil and platelet count, and some MDS patients with severe cytopenias are less symptomatic than others with more modest cytopenias. The bone marrow in MDS usually is normocellular or hypercellular for age, but 10% to 20% of cases are accompanied by a hypocellular marrow, and such cases of *hypoplastic* or *hypocellular MDS* may be difficult to distinguish from AA.

Premalignant conditions

Cytopenia is the sine qua non for any MDS diagnosis; however, there are individuals with blood cytopenias who do not meet the diagnostic criteria for MDS. Moreover, with the recent advent of inexpensive genomic sequencing technologies, it has also become clear that there are individuals with or without cytopenias who possess somatic clonal mutations known to be associated with MDS such as *DNMT3A*, *TET2*, and *ASXL1* but do not fully meet WHO criteria for a specific disease entity. Some, but not all, of these individuals go on to develop MDS or another hematologic neoplasm. Individuals with clonal mutations but without cytopenias progress to MDS at a rate similar to that observed with other premalignant conditions such as monoclonal gammopathy of undetermined significance (a precursor state for plasma cell dyscrasias) and monoclonal B-cell lymphocytosis (a precursor state for B-cell malignancies). Individuals with somatic mutations and cytopenias have a much higher rate of progression. The factors that determine progression are not currently well understood but are thought to involve progressive accumulation of genetic events (Figure 18-7). Because of their different prognoses, it is important to distinguish individuals with these premalignant conditions from those that meet diagnostic criteria for MDS (see video on MDS in online edition). In general, individuals diagnosed with these conditions should be monitored in a proactive fashion for the development of MDS or another hematologic disorder. The following terms have been proposed to describe individuals with cytopenias, clonal mutations seen in myeloid neoplasms, or both who do not meet formal WHO criteria for MDS:

ICUS

Individuals with single or multiple blood cytopenias that remain unexplained despite an appropriate evaluation (including bone marrow examination) and do not have a known associated clonal genetic alteration are identified as having ICUS. Individuals with ICUS may have cytopenias resulting from undiagnosed reactive condition, other non-neoplastic conditions, or a nonmyeloid neoplasm.

CHIP

Individuals known to have a clonal mutation associated with hematologic neoplasia present at a variant allele fraction of ≥ 2% but who do not yet meet diagnostic criteria for diagnosis of any hematologic neoplasm and do not have

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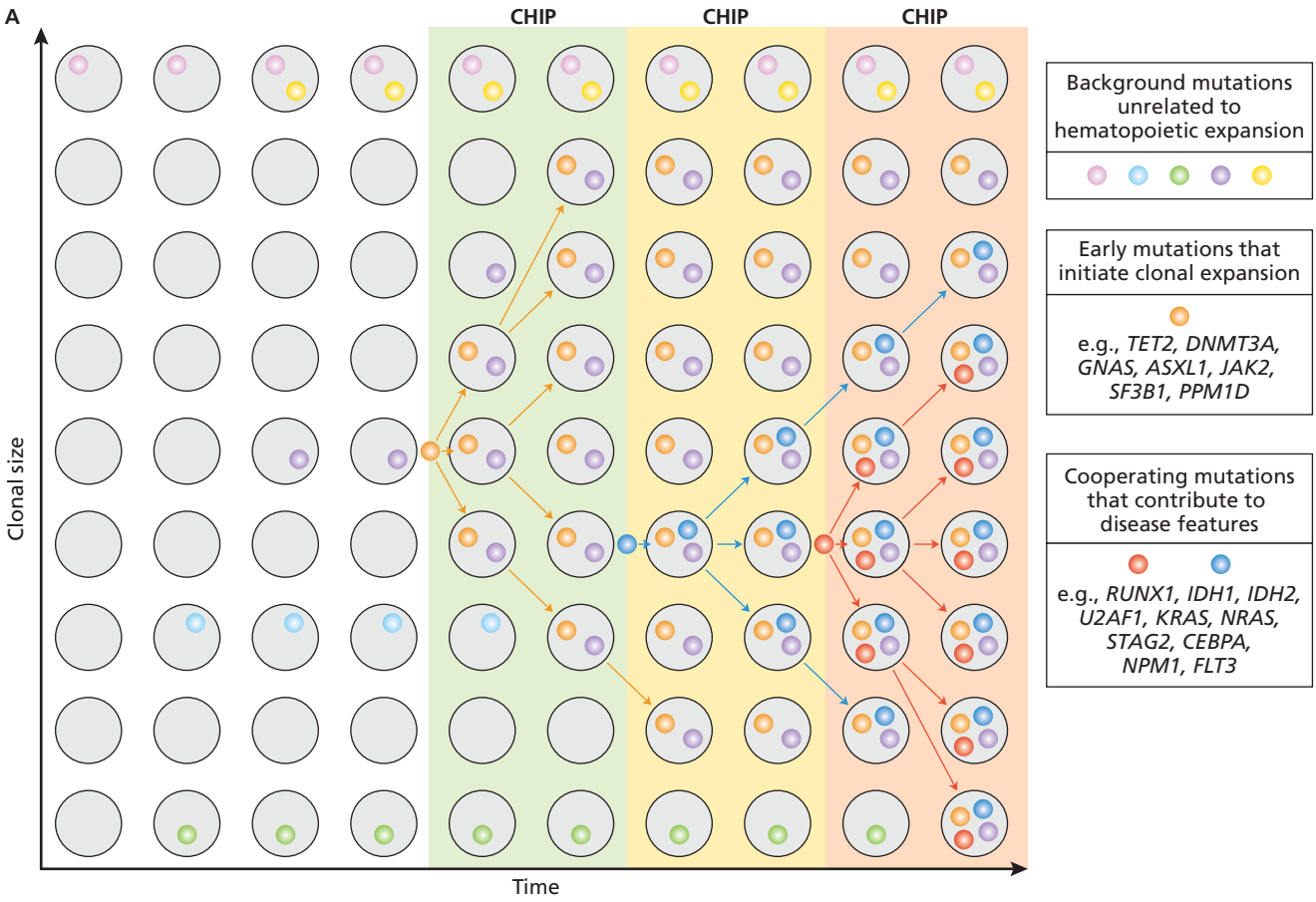
A
CHIP
CHIP
CHIP
Clonal size
Time
Background mutations unrelated to hematopoietic expansion
Early mutations that initiate clonal expansion
e.g., TET2, DNMT3A, GNAS, ASXL1, JAK2, SF3B1, PPM1D
Cooperating mutations that contribute to disease features
e.g., RUNX1, IDH1, IDH2, U2AF1, KRAS, NRAS, STAG2, CEBPA, NPM1, FLT3
B
Traditional ICUS
MDS by WHO 2016
"Nonclonal" ICUS
CHIP
CCUS
Lower-risk MDS
Higher-risk MDS
Clonality − + + + +
Dysplasia − − − + +
Cytopenias + − + + +
BM blast % <5% <5% <5% <5% <19%
Overall risk Very low Very low Low (?) Low High
Treatments Obs/BSC Observation Obs/BSC/GF Obs/BSC/GF/IMiD/IST HMA/HCST
Clonal cytopenias

Figure 18-7 Clonal hematopoiesis as a precursor state for hematological neoplasms. (A) A model for evolution from normal hematopoiesis to CHIP and then, in some cases, to MDS or AML. (B) The spectrum of clonal hematopoiesis, ICUS, and MDS. ICUS is a broad category that includes a heterogeneous group of individuals, some of whom have benign (nonclonal) hematopoiesis. Other patients with ICUS may have CHIP, differing only from lower-risk MDS by their lack of dysplasia and, currently, an undetermined disease risk. CHIP can also include patients with clonal hematopoiesis and nonmalignant causes of cytopenias (eg, immune cytopenias, liver disease, or nutritional deficiencies) that would not be considered to have ICUS because of the presence of a clone but may have a distinct natural history. BSC, best supportive care; GF, hematopoietic growth factor (eg, epoetin); HMA, hypomethylating agent (eg, azacitidine); IMiD, immunomodulatory drug (eg, lenalidomide); Obs, observation. Adapted from Steensma DP et al, *Blood*. 2015;126:9-16.

a clinically significant cytopenia are identified as having CHIP. The risk of CHIP increases with age, occurring in >10% of individuals over age 70 years with normal blood counts. Patients with prior exposure to chemotherapy or radiation appear to have higher rates of CHIP compared to a noncancer population. Individuals with CHIP have an

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increased risk of progression to a hematologic malignancy that is estimated at 0.5% to 1% per year. CHIP is also associated with an increase in all-cause mortality and an increased risk of cardiovascular events. This is currently attributed to the concept that the clonally derived cells promote inflammation in atherosclerotic plaques.

Clonal skewing of hematopoietic cells in apparently healthy individuals has also been described on the chromosome level using X-inactivation studies and analyses of genome-wide association study cohorts, which identified clonal mosaicism in the form of copy number changes. These changes, at chromosomal loci associated with hematological neoplasia (ie, 20q, 5q, 11q, and 17p), were associated with an increased risk of subsequent hematopoietic neoplasm.

CCUS

Individuals with a clonal mutation and 1 or more clinically meaningful unexplained cytopenias who do not meet WHO-defined criteria for a hematologic neoplasm. The progression risk for CCUS to overt MDS is higher than for ICUS or CHIP.

Classification

The WHO classification of MDS was revised in 2016 (Table 18-5). This update was intended to incorporate discovery of newly identified molecular features that have provided diagnostic and prognostic information as well as pathological insights into MDS disease biology.

Important classification factors in the current WHO MDS schema include the number of lineages with dysplasia in >10% of cells, the marrow and peripheral blood blast proportion (determined as a percentage of all nucleated bone marrow cells), whether or not <15% of erythroid precursor cells in the marrow are ring sideroblasts (or <5% if *SF3B1* mutation in present); whether or not Auer rods are present, and the presence of disease-defining cytogenetic abnormalities. Despite the discovery of recurrent mutations that can be identified in 80% to 90% of MDS patients, the WHO has incorporated only recurrent mutations in the spliceosome gene *SF3B1* into the diagnostic scheme of MDS with ring sideroblasts (MDS-RS). This is based on the clear link between ring sideroblasts and *SF3B1* mutation as well as association with a distinct gene expression profile and favorable prognosis. It is important to reiterate that the presence of MDS-related mutations in the absence of morphologic dysplasia, even in the presence of clinically significant cytopenias, is not diagnostic of MDS. Such individuals may still have an unrelated reactive cause of cytopenia and are best monitored as CHIP or CCUS. The WHO has grouped t-MDS with therapy-related AML because the outcome in such patients is poor, regardless of the blast count. Cases with both MDS and myeloproliferative features, such as leukocytosis or thrombocytosis, are classified in a separate overlap category of MDS/myeloproliferative neoplasms (MDS/MPNs), which includes chronic myelomonocytic leukemia (CMML) (defined by $\geq 1 \times 10^9$ blood monocytes per L) and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) (which requires a platelet count of $\geq 450 \times 10^9$/L). Although the WHO classification is useful diagnostically, it has only limited prognostic value, and other tools (described later) are more useful for risk stratification.

The observation that alkylating agents, topoisomerase inhibitors, and ionizing radiation predispose patients to both MDS and AML; evolution of MDS to AML in some patients over time; the existence of shared cytogenetic abnormalities, such as deletions or gains in all or parts of chromosomes 5, 7, 8, or 20; and shared common somatic mutations, such as *TET2* and *ASXL1*, imply a biologic continuum between MDS and AML. Whereas loss or gain of chromosomal material is common in MDS, chromosomal translocations are less common in MDS than in AML, and certain point mutations (eg, *FLT3*) common in AML are rarely seen in MDS. The so-called "good-risk" recurrent AML-associated translocations, t(8;21), t(15;17), and inv(16), are rare in patients with dysplasia, and the WHO classifies patients with these abnormalities as having AML regardless of the blast count or marrow dysplasia.

The natural history of MDS includes a risk of progression to treatment-refractory AML (~25% to 30% likelihood overall, with some subtypes of MDS such as MDS with excess blasts [MDS-EB-2] at much greater risk), but most patients with MDS do not develop AML. Instead, the majority of patients who are diagnosed with MDS die from complications of cytopenias, most commonly infections resulting from absolute neutropenia and neutrophil dysfunction, and less frequently thrombocytopenia-associated bleeding or anemia-exacerbated cardiovascular events. Because MDS are primarily diseases of older persons, some patients succumb to unrelated conditions that are common in the elderly; they die with MDS, rather than from MDS.

Epidemiology

Aging is the most important risk factor for development of MDS, in part because of the progressive accumulation of somatic mutations in HSCs across the human life span. Eventually, a mutation or combination of mutations can occur in such a way in a hematopoietic cell that its progeny acquires a growth and survival advantage and clonal hematopoiesis emerges. The expanded clone of cells is then at risk for acquiring additional mutations that increase its malignant potential.

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Table 18-5 2016 WHO classification of myelodysplastic syndromes and neoplasms

Name	Dysplastic lineages	Cytopenias*	BM/PB features	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia	1	1 to 2	Blasts: BM < 5%, PB < 1%, no Auer rods; <15%/<5%† ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia	2 or 3	1 to 3	BM < 5%, PB < 1%, no Auer rods; <15%/<5%† ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS				
MDS-RS with single lineage dysplasia	1	1 to 2	BM < 5%, PB < 1%, no Auer rods; <15%/<5%† ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia	2 or 3	1 to 3	BM < 5%, PB < 1%, no Auer rods; <15%/<5%† ring sideroblasts	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1 to 3	1 to 2	BM < 5%, PB < 1%, no Auer rods; del(5q) alone or with 1 additional abnormality except -7 or del(7q); no ring sideroblasts	del(5q) alone or with 1 additional abnormality except -7 or del(7q)
MDS-EB				
MDS-EB-1	0 to 3	1 to 3	BM 5% to 9% or PB 2% to 4%, no Auer rods; no ring sideroblasts	Any
MDS-EB-2	0 to 3	1 to3	BM 10% to 19% or PB 5% to 19% or Auer rods; no ring sideroblasts	Any
MDS, unclassifiable				
With 1% blood blasts	1 to 3	1 to 3	BM < 5%, PB = 1%‡ no Auer rods; no ring sideroblasts	Any
With single lineage dysplasia and pancytopenia	1	3	BM < 5%, PB < 1%, no Auer rods; no ring sideroblasts	Any
Based on defining cytogenetic abnormality	0	1 to 3	BM < 5%, PB < 1%, no Auer rods; <15%§	MDS-defining abnormality¶
Refractory cytopenia of childhood	1 to 3	1 to 3	BM < 5%, PB < 2%, no ring sideroblasts	Any

Adapted from Arber DA et al, *Blood*. 2016;127(20):2391-2405.

PB, peripheral blood.

*Cytopenias defined as hemoglobin, <10 g/dL; platelet count, <100 × 10^9/L; and absolute neutrophil count, <1.8 × 10^9/L. PB monocytes must be <1 × 10^9/L.

†If *SF3B1* mutation is present.

‡One percent PB blasts must be recorded on at least 2 separate occasions.

§Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia and are classified as MDS-RS with single lineage dysplasia.

¶MDS-defining abnormalities (by conventional cytogenetics): −7 or del(7q), t(11;16)(q23;p13.3), −5 or del(5q), t(3;21)(q26.2;q22.1), i(17q) or t(17p), t(1;3)(p36.3;q21.1), −13 or del(13q), t(2;11)(p21;q23), del(11q), inv(3)(q21q26.2), del(12p) or t(12p), t(6;9)(p23;q34), del(9q), idic(X)(q13), or complex karyotype (3 or more chromosomal abnormalities involving 1 or more of the above).

The median age at diagnosis of MDS in the United States and Europe is ~70 years. Accurate estimates of the incidence of MDS have been difficult to obtain because MDS cases have not historically been captured by cancer registries and many elderly patients with mild cytopenias are incompletely evaluated. However, current registry and claims-based algorithms suggest there are 30,000 to 40,000 new cases of MDS diagnosed per year in the United States. Most patients have lower-risk disease at the time of initial diagnosis.

MDS diagnoses are rare in the pediatric age group and represent ~5% of hematologic malignancies in patients <18 years of age. When MDS does arise in children, the diagnosis is frequently associated with Down syndrome, congenital marrow failure syndromes, or germline defects of DNA repair, such as Li-Fraumeni syndrome or Bloom syndrome. Children with Shwachman-Diamond syndrome, congenital neutropenia, or FA are at markedly increased risk of developing MDS (see Chapter 15). In all of these inherited conditions, MDS arises in the context of hematopoietic deficits and typically presents in late childhood or in adolescence.

In the majority of adult patients with MDS, the etiology is unknown, and there is no specific predisposing factor identifiable other than advanced age. However, a subset of patients with MDS, AML, or MPN, particularly those with

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a family history of related disorders/cytopenias or with t-MDS, may have a familial syndrome with inherited germline predisposition. The 2016 revision of the WHO classification now identifies these cases as myeloid neoplasms with germline predisposition. Examples of these include myeloid neoplasms associated with germline mutations in transcription factor genes *RUNX1* and *GATA2*, the RNA helicase *DDX41* or the centriole distal appendage component *ANKRD26*. Germline *RUNX1* and *ANKRD26* mutations are associated with a prodrome of thrombocytopenia. *DDX41* mutations have no associated prodrome and unlike other germline predisposition syndromes, tend to present at older age. *GATA2* mutations are sometimes nonsyndromic but can be associated with mycobacterial infections, lymphedema, and monocytopenia (MonoMAC syndrome).

KEY POINTS

- MDS is characterized by ineffective hematopoiesis, leading to peripheral blood cytopenias. The marrow is often hypercellular for age.
- Anemia (usually macrocytic) is the most common cytopenia associated with MDS. Functional defects in neutrophils and platelets can exacerbate the risk of infection from neutropenia or bleeding from thrombocytopenia.
- Aging and exposure to alkylating agents, topoisomerase II inhibitors, or ionizing radiation are all risk factors for developing MDS.
- MDS is rare in children, and when it occurs is often associated with congenital marrow failure syndromes.
- An increasing number of germline mutations such as those in *RUNX1* and *GATA2* are associated with a subsequent risk for MDS development. *RUNX1* mutations are also associated with thrombocytopenia and are often mistaken for immune thrombocytopenic purpura until MDS develops or additional family members are diagnosed.
- The 2016 WHO classification of MDS is the current standard, but it should be used in conjunction with risk stratification tools to assess prognosis.

Diagnostic evaluation

After a medical history and physical examination, the diagnosis of MDS is readily established in most patients by a complete blood count, careful review of the blood smear, bone marrow examination, and basic laboratory tests to rule out other disorders that mimic MDS. Vitamin B_{12} and folate deficiency, viral infection, copper deficiency, alcohol use disorder, and adverse effects of medication (eg, antimetabolites such as methotrexate or azathioprine) need to be excluded as should other causes of anemia such as iron deficiency and thyroid disorders. Cytopenias should be persistent (at least 4 to 6 months in duration) and cannot be attributable to other underlying conditions. The pathologic diagnosis of MDS currently emphasizes morphologic criteria including dysplastic features in the peripheral blood and >10% of bone marrow precursor cells in 1 or more lineages—erythroid, myeloid, and/or megakaryocytic (Figure 18-8). Additionally, an increased blast count (5% to 19%) or presence of an MDS-associated karyotype is also diagnostic.

Presentation with anemia alone in MDS is relatively uncommon. Although patients with MDS often seek medical attention because of symptoms related to cytopenias, many patients are asymptomatic at diagnosis and are discovered to have MDS only when a complete blood count is performed as a screening test or to evaluate another condition.

Oval macrocytic red blood cells, hypogranular and hypolobulated granulocytes, and giant or hypogranular platelets can be identified in the peripheral blood of many patients with MDS. Bilobated hyposegmented neutrophils in MDS resemble those seen in the clinically inconsequential congenital Pelger-Huët anomaly and are referred to as *Pelgeroid* or *pseudo–Pelger-Huët cells*. Peripheral blood smears may be highly suggestive of the diagnosis but are never conclusive by themselves. A marrow aspirate is essential to establish definitively a diagnosis of MDS, and the bone marrow core biopsy provides complementary information on cellularity and architecture, megakaryocyte morphology, and the presence of fibrosis. Increased reticulin fibrosis in the marrow at diagnosis are seen in approximately 5% to 10% of MDS cases and are associated with poor prognosis.

The bone marrow biopsy in MDS usually demonstrates hypercellularity, which, in the setting of cytopenias in the peripheral blood, indicates ineffective hematopoiesis. On the marrow aspirate, megaloblastoid red blood cell precursors with asynchronous maturation of the nucleus and the cytoplasm are usually evident, and multinucleated erythroid precursors are common (Figure 18-8). Ring sideroblasts, which are erythroid precursors with iron-stuffed mitochondria (stored as mitochondrial ferritin, a unique type of ferritin) surrounding at least 1/3 of the nucleus, may be identified via the Prussian blue reaction, and often there is predominance of immature myeloid cells and dysplastic granulocytic precursors. Dysplastic megakaryocytes may be smaller or larger than normal and may be hypolobated or hyperlobated. Dysplastic features in all lineages can include nuclear and cytoplasmic blebs and misshapen nuclei.

Cytogenetic studies can further support a diagnosis of MDS and are important for prognosis and treatment decisions (Tables 18-6 and 18-7). Standard cytogenetic assessment is preferred, but in a small percentage of cases,

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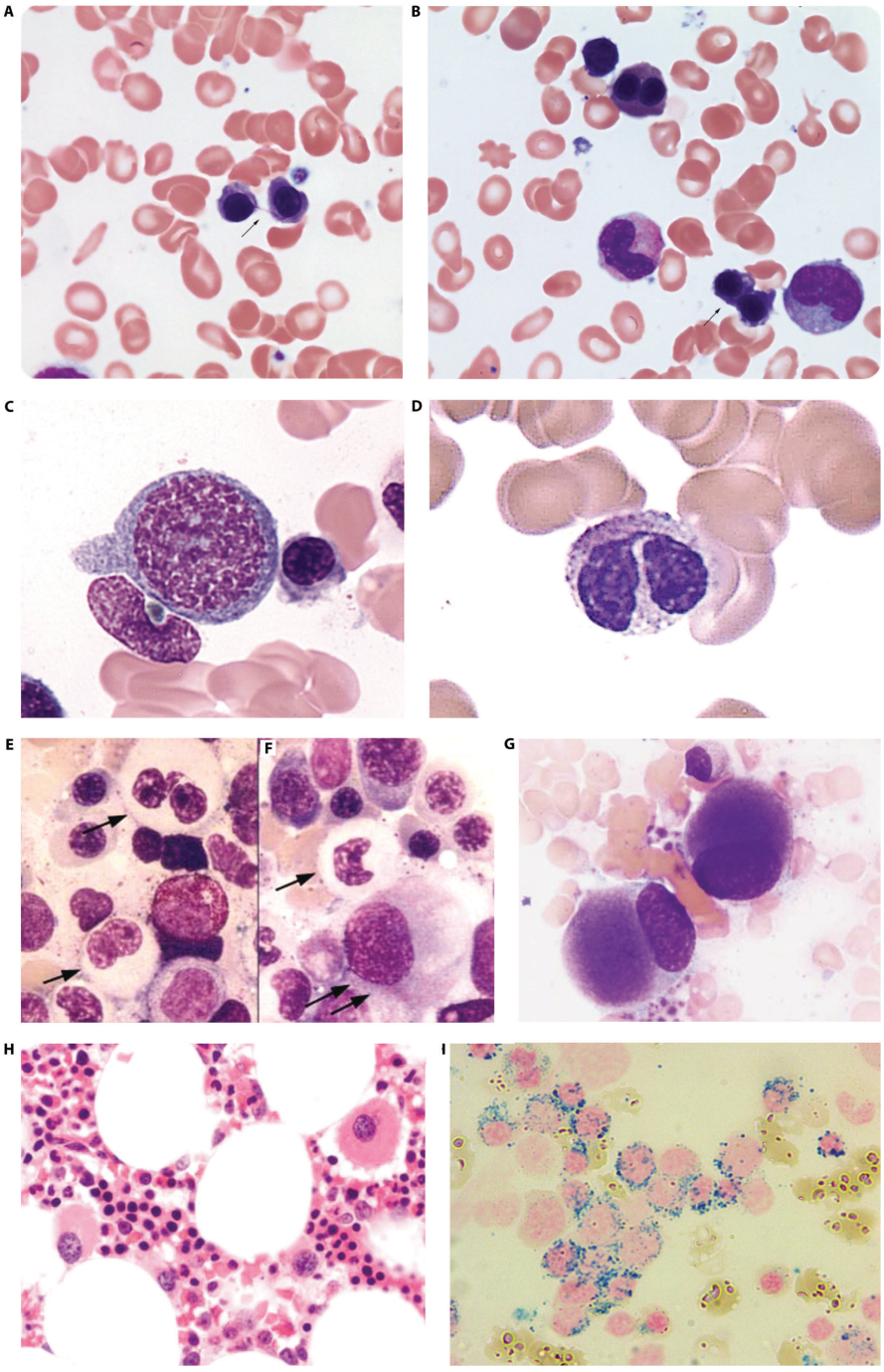

Figure 18-8 Typical blood and marrow cell morphology in patients with MDS. (A and B) Multinucleated erythroid precursors (arrows); the cells in panel a have a visible cytoplasmic bridge, which is uncommonly observed. Wright-Giemsa–stained marrow aspirate. Source: American Society of Hematology (ASH) Image Bank (imagebank.hematology.org), #00030315. (C) Megaloblastoid erythroid cell maturation (nuclear-cytoplasmic dyssynchrony). The chromatin pattern of these cells is fine, suggesting relative immaturity, whereas the lightening of the cytoplasm indicative of early hemoglobinization is an event typically associated with later stages of maturation. Source: ASH Image Bank #00002571. (D) Hypolobated neutrophil (pseudo–Pelger-Huët cell) found in the peripheral blood of a patient with refractory cytopenias with multilineage dysplasia. The cell vaguely resembles a pince-nez, a style of eyeglasses supported without earpieces popular in the 19th century. Source: ASH Image Bank #00002117. (E and F) Hypogranular neutrophils (arrows). These would be expected to have poor bactericidal activity. The double arrow in panel F indicates a small, dysplastic megakaryocyte. Source: ASH Image Bank #00001435. (G and H) Micromegakaryocytes in a Wright-Giemsa–stained aspirate (G) and hematoxylin-eosin–stained core trephine biopsy specimen (H). These may have an eccentric, hypolobulated, or round nucleus. These images are from a patient with 5q– syndrome. Source: ASH Image Bank #00001446 (H) and #00001448 (G). (I) Ring sideroblasts (a Prussian blue reaction on a marrow aspirate, seen at low power magnification and counterstained with neutral red). Source: ASH Image Bank (image 00001157).

fluorescence in situ hybridization (FISH) analysis with probes directed toward chromosomes frequently rearranged in MDS (eg, 5, 7, 8, 20) reveals specific chromosomal translocations and losses or gains of DNA segments that were not detected with standard cytogenetic methods. FISH is helpful in cases in which 20 or more metaphases cannot be obtained, but the yield of FISH is low if karyotyping is successful.

Flow cytometric analysis of the bone marrow can be helpful to evaluate patients with suspected MDS. Flow cytometry can demonstrate abnormal differentiation patterns or aberrant antigen expression which may help confirm a diagnosis of MDS and/or rule out alternative diagnoses. Flow cytometry can also be helpful to detect clonal expansion of large granular lymphocytes, which may predict response to IST. Because accurate

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Table 18-6 IPSS-R for MDS (2012 version)

Parameter	IPSS-R categories and associated scores				
Cytogenetic risk group	Very good	Good	Intermediate	Poor	Very poor
	0	1	2	3	4
Marrow blast proportion	<2%	2% to <5%	5% to 10%	>10%	
	0	1	2	3	
Hemoglobin	≥10 g/dL	8 to <10 g/dL	<8 g/dL		
	0	1	1.5		
Absolute neutrophil	$\geq 0.8 \times 10^9$/L	$<0.8 \times 10^9$/L			
Count	0	0.5			
Platelet count	$\geq 100 \times 10^9$/L	50×10^9 to 100×10^9/L	$<50 \times 10^9$/L		
	0	0.5	1		

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454-2465.

Possible range of summed scores: 0 to 10.

Table 18-7 MDS cytogenetic risk stratification system used in the IPSS-R

	Updated cytogenetic classification for use in IPSS-R (N = 7012)			
Risk group	Included karyotypes	Median survival, y	25% of patients to AML, y	% of patients in this group
Very good	del(11q), -Y	5.4	NR	4
Good	Normal, del(20q), del(5q) alone or with 1 other anomaly, del(12p)	4.8	9.4	72
Intermediate	+8, del(7q), i17q, +19, any other single or double abnormality not listed, 2 or more independent clones	2.7	2.5	13
Poor	Abnormal 3q, -7, double abnormality includes -7/del(7q), complex with 3 abnormalities	1.5	1.7	4
Very poor	Complex with more than 3 abnormalities	0.7	0.7	7

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454-2465.

NR, not reached.

classification according to WHO criteria is based, at least in part, on bone marrow morphology, flow cytometry is best interpreted in the context of the appearance of the marrow morphology. Specifically, flow cytometric enumeration of marrow blasts should not replace a manual differential from the marrow aspirate, because it is subject to technical artifacts.

Increasingly, molecular profiling is playing an important role in evaluation of patients suspected of having MDS, especially in ambiguous cases with bland morphology but no other explanation for cytopenias. Almost all patients with MDS have a somatic mutation detectable in 1 of the commonly mutated MDS-associated genes, so the negative predictive value of a normal result on an MDS mutation panel is high, and another cause for cytopenias should be carefully sought in such cases. However, as mentioned earlier, because clonal hematopoiesis is common in healthy older people, detection of a mutation in patients with a normal karyotype and without morphological changes of dysplasia should be interpreted with caution and is not diagnostic of MDS. Molecular profiling can also aid in prognostic assessment and decisions about stem cell transplant. Detection of an *SF3B1* mutation, for instance, would support a diagnosis of MDS-RS rather than a congenital sideroblastic anemia or reactive cause of sideroblastic anemia, while the finding *TP53* mutation makes stem cell transplant less likely to be successful. Finally, additional genetic screening for mutations associated with inherited predisposition syndromes should be strongly considered in patients with a family history of hematologic malignancies and familial cytopenias, in patients with t-MDS, or in younger patients with MDS. Such testing should ideally be done on constitutional tissue such as skin fibroblasts to confirm the germline nature of such alterations and avoid false negatives associated with peripheral blood somatic mosaicism.

Overall, the diagnosis of MDS is evolving toward the approach used in AML, in which morphologic, cytogenetic, and flow cytometric data are assessed together to make an accurate diagnosis and determine the optimal

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treatment. This strategy will become increasingly important as biologically distinct subsets of MDS patients who respond to specific therapies are defined.

KEY POINTS

- Complete blood counts, marrow aspirate and core biopsy, blood and marrow morphology, and cytogenetic testing are key. Next-generation sequencing is increasingly used to establish a diagnosis of MDS.
- Flow cytometry may provide complementary information but cannot be used to establish a diagnosis of MDS in the absence of marrow morphology. Blast counts should be based primarily on a manual assessment of the marrow aspirate by an experienced morphologist.
- Vitamin deficiencies, viral infections, alcohol use disorder, systemic illnesses with autoimmunity and medication effects can cause cytopenias and dysplastic changes in blood cells and need to be excluded.
- Molecular abnormalities are present in most cases of MDS, and molecular testing can be used as a diagnostic tool and as an aid in prognostic assessment.

Prognosis

In 1997, the International Prognostic Scoring System (IPSS) was developed to help stratify patients with MDS by their risk of disease progression to acute leukemia and death. In 2012, a revised version of the IPSS (IPSS-R) was published, based on analysis of >7000 patients from more than 10 countries (Tables 18-6, 18-7, and 18-8). The IPSS-R includes a broader range of cytogenetic abnormalities and weighs cytogenetic findings more heavily than other variables. Degree of cytopenias is given more weight in the IPSS-R than in the IPSS, and blast cutoffs are different. Like the original IPSS, the IPSS-R is most valid in patients with de novo MDS and only at the time of diagnosis. In addition, other prognostically important variables, such as the presence of comorbid conditions and the patient's performance score, molecular genetic findings, and the kinetics of clonal evolution and disease progression, are not accounted for by the IPSS-R.

More than 80% of patients with MDS have at least 1 somatic mutation detectable in hematopoietic cells (see the following "Biology" section). Several of these mutations have IPSS-independent prognostic significance. For instance, patients with mutations in *TP53*, *ETV6*, *RUNX1*, *ASXL1*, or *EZH2* have a greater risk of leukemia progression or death than would be predicted by the IPSS, and patients with IPSS low-risk disease who harbor one of these mutations have an outcome more similar to IPSS intermediate-1–risk disease. New prognostic systems are being developed to incorporate molecular abnormalities into the IPSS-R.

Myelodysplastic/myeloproliferative neoplasms

The category of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) is separate from MDS and includes disorders with overlapping dysplastic and proliferative features such as CMML; atypical chronic myeloid leukemia, BCR-ABL1 negative; and juvenile myelomonocytic leukemia. The category of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) is separate from MDS and includes disorders with overlapping dysplastic and proliferative features such as CMML; atypical chronic myeloid leukemia, BCR-ABL1 negative; juvenile myelomonocytic leukemia; MDS/MPN-RS-T; and MDS/MPN, unclassifiable (MDS/MPN-U).

CMML has been subdivided into 2 groups based on molecular and clinical differences: proliferative-type CMML (white blood cell count ≥13 × 109/L) and dysplastic type CMML (white blood cell count <13 × 109/L). The percentage of blasts plus monocytes in the blood and bone marrow has prognostic significance in these patients. The 2016 WHO classification defines 3 prognostic groups for CMML: CMML-0, for patients with less than 2% peripheral blood blasts and less than 5% bone marrow blasts, CMML-1 for patients with 2% to 4% peripheral blood blasts and/or 5% to 9% bone marrow blasts, and CMML-2 for patients with 5% to 19% peripheral blood

Table 18-8 Survival and AML progression risk with the 2012 IPSS-R for MDS

Risk group	Points	% patients (N = 7012; AML data on 6485)	Median survival, y	Median survival for patients under 60 y	Time until 25% of patients develop AML, y
Low	2.0 to 3.0	38%	5.3	8.8	10.8
Very low	0 to 1.5	19%	8.8	Not reached	Not reached
Intermediate	3.5 to 4.5	20%	3.0	5.2	3.2
High	5.0 to 6.0	13%	1.5	2.1	1.4
Very high	>6.0	10%	0.8	0.9	0.7

Adapted from Greenberg PL et al, *Blood*. 2012;120:2454-2465.

IPSS-R (see http://www.mds-foundation.org/ipss-r-calculator/).

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blasts, 10% to 19% bone marrow blasts, and/or the presence of Auer rods. Mutations in the following genes are frequently found in CMML: *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *NRAS*, and *CBL*. The management of CMML depends on the characteristics of the patient's disease and can include observation in patients with CMML-0. For patients with CMML-1 and CMML-2, hypomethylating agents (HMAs), have some modest efficacy (see section Hypomethylating agents [DNA methyltransferase inhibitors]). Higher-risk patients are candidates for hematopoietic stem cell transplantation.

KEY POINTS

- The IPSS was previously the most widely used risk stratification system in MDS but was revised in 2012 (IPSS-R) to include a broader range of karyotypes and other modifications. The IPSS-R is now broadly used to prognosticate for MDS patients.
- Factors associated with poorer outcomes in MDS include advanced age, comorbid conditions and poor performance score, increased marrow and blood blasts, more severe cytopenias and transfusion dependence, higher-risk karyotypes (eg, a complex karyotype or monosomy 7), and the presence of certain mutations (eg, *TP53* or *RUNX1*).
- Patients with MDS/MPN are a heterogenous group of disorders with features of dysplasia and proliferation. Treatment is based on individual clinical and molecular features.

Biology

Chromosome and molecular biology

Approximately 1/2 of patients with de novo MDS and most patients with t-MDS have cytogenetic abnormalities detectable on routine metaphase karyotyping. Cytogenetic results have independent prognostic significance (Table 18-7). One particular clonal abnormality involving interstitial or terminal deletion of part of the long arm of chromosome 5 (5q–) has received a great deal of attention because patients with deletions of chromosome 5q preferentially respond to lenalidomide therapy (see section "Treatment of MDS" later in this chapter). Haploinsufficiency of a 5q-encoded ribosomal protein, RPS14, contributes to defective erythropoiesis, just as germline mutations of ribosomal components contribute to DBA (see the section on DBA in Chapter 15). As originally described, the 5q– syndrome is associated with erythropoietin-refractory macrocytic anemia, dyserythropoiesis, normal or increased platelet count, giant platelets, hypolobated megakaryocytes, variable neutropenia, female predominance, prolonged survival, and a low rate of leukemic transformation. It is important to differentiate the 5q– syndrome from other myeloid disorders in which chromosome 5q deletions are found as they are not biologically the same. Patients with the del(5q) without the characteristic clinical and morphologic features of 5q– syndrome may have a more aggressive clinical course and shorter survival than those with the classic syndrome, although they still may respond to lenalidomide treatment. The extent of the chromosome 5q deletion in MDS also has prognostic value, with small interstitial deletions associated with better outcomes than larger deletions.

The clinical and genetic heterogeneity found in MDS and the typical advanced age at disease onset support the idea that multiple cooperating genetic lesions contribute to leukemogenesis. Unlike AML and MPNs, which frequently demonstrate chromosomal translocations, gains and losses of entire chromosomes or of large DNA segments are more common in MDS. In recent years, high-throughput resequencing techniques revealed recurrent point mutations in more than 40 different genes, some of which are shared with AML and other neoplasms (Figure 18-9). These techniques also demonstrate that the majority of cells in the marrow are clonal, even in lower-risk MDS with <5% blasts.

Activating mutations in proto-oncogenes such as *NRAS*, *FLT3*, and *JAK2* are detected in many cases of AML or MPN but are uncommon in MDS. Although *RAS/RAF* pathway mutations are common in the MDS-MPN overlap syndromes of chronic myelomonocytic leukemia and juvenile myelomonocytic leukemia, these mutations are rare in MDS without MPN features and usually are found only after progression to acute leukemia. These data suggest that aberrant activation of signal transduction pathways may not be a major mechanism of aberrant cell growth and clonal dominance in early MDS, which distinguishes these diseases from other myeloid malignancies.

The *TP53* tumor suppressor gene, which regulates cell cycle progression, DNA repair, and apoptosis, is mutated in 5% to 10% of MDS cases overall and in a higher proportion of t-MDS. *TP53* mutation is often associated with a complex karyotype and has a strong negative prognostic significance. Mutations in the gene *PPM1D*, which encodes a negative regulator of p53, are also enriched in patients with t-MDS (15% to 20% of cases) and are associated with exposure to DNA-damaging agents. *RUNX1* point mutations also are relatively common in patients with t-MDS.

Mutations in genes altering DNA methylation and chromatin remodeling are common in MDS. *TET2*

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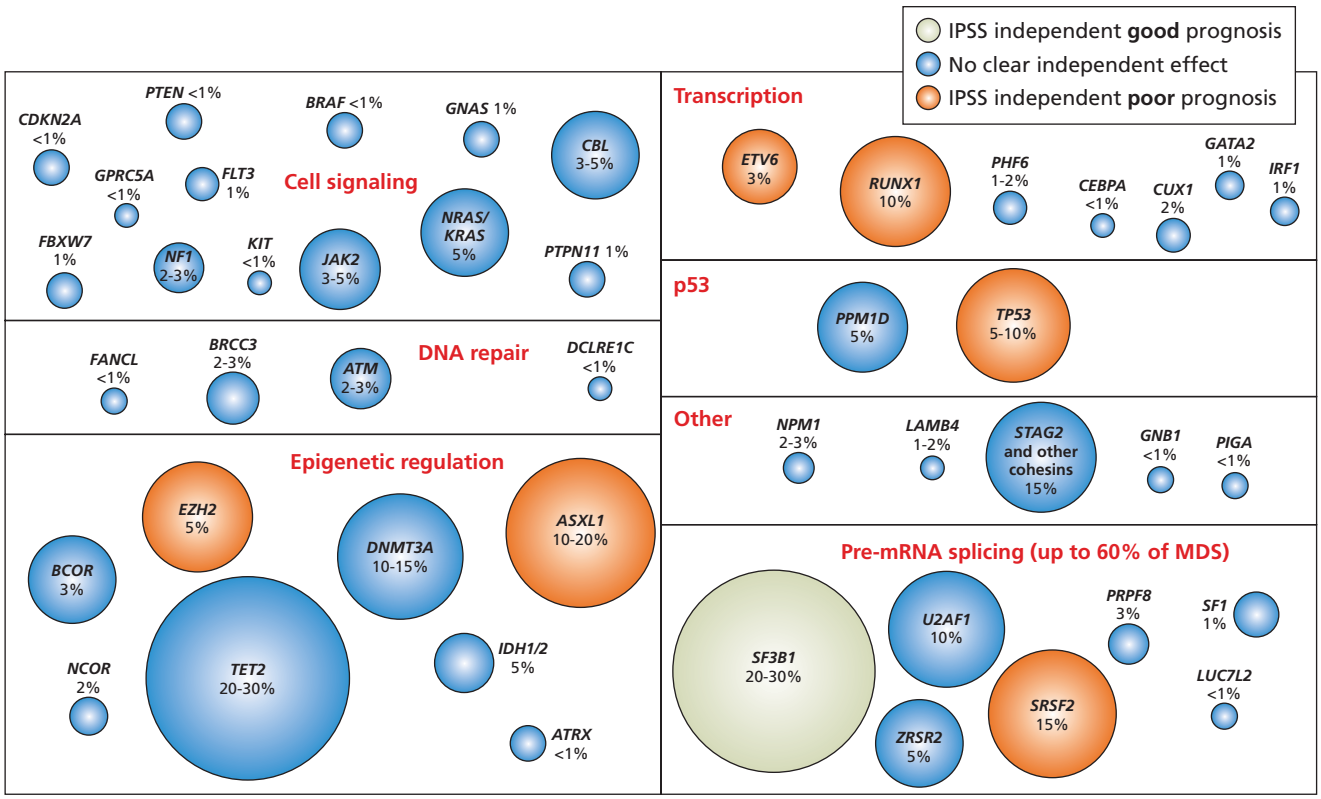

Figure 18-9 Recurrent somatic mutations in MDS, including approximate frequency of the most common recurrent somatic mutations in MDS and their prognostic significance. Some mutations influence the phenotype and are therefore more common in specific subtypes of MDS; for instance, *SF3B1* mutations are found in up to 80% of patients with MDS-RS, and *SRSF2* mutations are more common in MPN/MDS overlap syndromes such as chronic myelomonocytic leukemia. Mutation frequencies and their prognostic significance are derived from Bejar R et al, *N Engl J Med*. 2011;364:2496-2506; Haferlach T et al, *Leukemia*. 2014;28: 241-247; and Papaemmanuil E et al, *Blood*. 2013;122:3616-3627. The negative prognostic impact with *SRSF2* mutations is from Thol F et al, *Blood*. 2012;119:3578-3584.

mutations, for example, are present in 20% to 30% of patients, and recurrent mutations are also found in *EZH2*, *IDH1* and *IDH2*, and *ASXL1*. Another class of recurrent mutations in MDS are genes that encode components of the spliceosome and alter RNA splicing, especially *SF3B1*, which is present in the majority of patients with MDS-RS. Other common mutations in spliceosome components include *SRSF2* and *U2AF1*. Mutations in genes such as *STAG2* or *RAD21* that encode components of the cohesin protein complex, which regulates the separation of sister chromatids during cell division, are found in up to 20% of MDS.

Patients who develop t-MDS secondary to exposure to mutagenic or carcinogenic agents almost always have chromosomal abnormalities. t-MDS is most commonly associated with previous treatment with alkylating agents or exposure to ionizing radiation, and these cases frequently demonstrate losses involving chromosomes 5 or 7. The latency period for t-MDS arising after alkylating agent therapy is typically 3 to 7 years. Patients treated with epipodophyllotoxins (eg, etoposide) can develop specific translocations involving the breakpoint at 11q23; the latency period between exposure and MDS/AML development is typically 1 to 3 years. These 11q23 translocations lead to transcription of a fusion protein involving the mixed-lineage leukemia (*MLL*) gene. Translocations and inversions of 3q21/3q26 can arise after etoposide treatment and involve rearrangement of the *MDS1-EVI1* (*MECOM*) genes; such patients often have a normal or elevated platelet count at the time of diagnosis and have a grim prognosis.

KEY POINTS

- One-half of patients with de novo MDS and most patients with secondary MDS present with a clonal cytogenetic abnormality.

Key Points continue

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KEY POINTS *(continued)*

- 5q– syndrome has a relatively benign prognosis, but not all patients with del(5q) have 5q– syndrome. Deletion of *RPS14*, a gene on chromosome 5q that encodes a ribosomal subunit, contributes to the erythropoietic defect in del(5q) MDS and links del(5q) MDS to DBA, which is caused by heterozygous germline mutations in genes, such as *RPS19*-encoding ribosomal proteins.
- Patients with t-MDS who have been exposed to alkylating agents or ionizing radiation usually have abnormalities of chromosomes 5 and 7, whereas those who have been exposed to epipodophyllotoxins usually have abnormalities of chromosome 11q23.
- More than 40 genes are recurrently mutated in patients with MDS. Those with IPSS-independent prognostic value include *SF3B1*, *EZH2*, *TP53*, *RUNX1*, *ASXL1*, and *ETV6*.

Treatment of MDS

With the exception of allogeneic HSCT, no therapeutic options in MDS have demonstrated curative potential. However, 6 medications have specific FDA approval for MDS-related indications (azacitidine, decitabine, lenalidomide, luspatercept, and an oral combination of decitabine and cedazuridine) and these drugs offer benefit to a subset of patients. Advanced age, the presence of comorbidities, and a lack of a suitable donor limit the availability of allogeneic HSCT, but use of reduced-intensity conditioning approaches and alternative stem cell sources (eg, umbilical cord blood and haploidentical donors) are expanding the roster of potentially eligible patients. Therefore, patients with MDS who are potential candidates for transplantation should be evaluated early in the disease course by a physician with expertise in stem cell transplantation. In many transplant centers, reduced-intensity stem cell transplantation is routinely performed for patients into their 70s; therefore, age alone should not be considered an exclusion.

Goals of MDS therapy for an individual patient depends in part on the stage of disease and includes symptom control, reduction of transfusion needs, delay of disease progression, and extension of survival. Prognostic systems such as the IPSS-R, supplemented by molecular testing, allow clinicians to incorporate clinicopathological risk factors for death and disease progression into therapeutic decisions.

Hypomethylating agents (DNA methyltransferase inhibitors)

Cytidine residues in mammalian DNA can be methylated, and DNA methylation is a dynamic process that affects transcription rates. Methylated cytidine residues cluster in cytosine-phosphate-guanine islands, which are located near the promoter regions of many genes. When these regions are hypermethylated, expression of nearby genes is silenced, and this represents a mechanism for regulating gene transcription. DNA methyltransferase 1 (DNMT1) is the enzyme responsible for maintenance of cytidine methylation patterns, and the aza-substituted cytosine nucleoside analogs azacitidine and decitabine can inhibit DNMT1 by incorporating into RNA or DNA and irreversibly binding to this enzyme, resulting in generalized hypomethylation of DNA and reversal of gene silencing. Although these epigenetic changes occur *in vitro* in cells exposed to DNMT1 inhibitors, it is not clear whether these epigenetic effects are responsible for the clinical activity of azacitidine or decitabine in MDS or whether other biologic effects (eg, DNA damage) also play a role.

Azacitidine is the first and, as of this writing, remains the only medication that has been shown in a randomized trial to improve survival in higher-risk MDS patients. In a multicenter trial (AZA-001), 358 patients with IPSS intermediate-2 or high-risk MDS were randomized to receive either azacitidine 75 mg/m^2 subcutaneously for 7 consecutive days every 28 days or conventional care. The median survival time was 24 months in patients receiving azacitidine versus 15 months in patients receiving conventional care. Although the complete response rate in the azacitidine-treated group was a modest 17%, subsequent analysis demonstrated that a complete response was not necessary for patients to achieve a survival benefit; however, it is unclear whether stable disease alone or minor hematologic improvements are beneficial. Azacitidine is approved for intravenous administration and subcutaneous dosing. Intravenous administration avoids injection site reactions but requires either central or peripheral venous access.

Decitabine is also active in MDS, but a European multicenter study designed to show a survival benefit with decitabine in MDS was negative. The overall survival of the control arm in that study (8 months) suggests that a different population was enrolled compared to AZA-001.

Clinical response to hypomethylating agents (HMA) may be delayed, and an adequate therapeutic trial of either agent requires at least 4 to 6 treatment cycles. Although the initial FDA approval of decitabine was for a regimen of 15 mg/m^2 administered every 8 hours for 9 doses intravenously (in a hospital-based setting), the most commonly used regimen in clinical practice is 20 mg/ m^2 intravenously once daily for 5 consecutive days, repeated every 4 to 6 weeks. In a multicenter study of this 5-day decitabine regimen, 17% of patients achieved a complete response,

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15% achieved a marrow response, and 18% experienced hematologic improvement, similar to the response rates observed with azacitidine therapy.

An oral combination of decitabine and cedazuridine has recently been FDA-approved for CMML and MDS with intermediate-1, intermediate-2, and high-risk IPSS scores. This approval was based on 2 open-label, randomized, crossover trials in MDS and CMML that showed that decitabine and cedazuridine were equivalent to decitabine 20mg/m^2 in terms of pharmacokinetic exposure and safety. In each trial, patients were randomized 1:1 to receive 35 mg decitabine and 100 mg cedazuridine orally in cycle 1 and decitabine 20 mg/m^2 intravenously in cycle 2 or the reverse sequence. Both oral and intravenous decitabine preparations were administered once daily on days 1 through 5 of a 28-day cycle. Starting with cycle 3, all patients received decitabine and cedazuridine orally once daily on days 1 through 5. Comparison of disease response was not possible because all patients received oral decitabine and cedazuridine from cycle 3 on. However, complete remission (CR) rates were similar to prior IV decitabine studies as were measures of decitabine exposure, DNA demethylation, and safety.

The most common adverse events associated with HMAs are neutropenia and thrombocytopenia, which often improve over time with continued treatment as the MDS clones are suppressed and normal hematopoiesis recovers. Therapy should be continued as long as response is retained. Thus far, no therapy has been demonstrated to improve survival for patients with lower-risk MDS, though HMAs can improve peripheral counts and reduce transfusion needs in a minority of such patients.

Early data suggest that the addition of venetoclax to HMA may improve response rates in treatment-naïve, high-risk MDS. A phase 1b study of 7 days of azacitidine 75mg/m^2 and venetoclax reported an overall response rate (ORR) of 77%, including a CR rate of 42%. In this study, the recommended phase 2 dose of venetoclax was 400 mg for 14 days/28-day cycle in combination with azacitidine, Hematologic toxicity was common, including neutropenia in 51% of patients.

Once HMAs fail, the prognosis is grim, with a median survival <6 months. Patients relapsed on or refractory to HMAs should be referred for HSCT or enrolled in clinical trials whenever feasible. The addition of venetoclax to HMAs has demonstrated some clinical activity in patients with HMA failure, but the use of venetoclax is not standard in this population. One small retrospective study reported an ORR of 59% in 44 patients with MDS, including an ORR of 44% in 16 patients with HMA failure defined as a lack of response after 4 cycles of HMA, progressive disease after at least 2 cycles of HMA, or progressive disease after achieving a response. Responses are also seen in some patients with low-dose cytarabine or clofarabine.

Other agents

Lenalidomide, an analog of thalidomide, is FDA-approved for treatment of transfusion-dependent anemia caused by low- or intermediate-1-risk MDS associated with a deletion 5q abnormality with or without additional cytogenetic abnormalities. The primary mechanism of lenalidomide is via modulation of E3 ubiquitin ligase cereblon activity, which results in altered degradation rate of casein kinase 1, a serine-threonine kinase that is encoded on chromosome 5q and which modulates Wnt/β-catenin signaling.

After phase 1 testing suggested a high response rate in del(5q) MDS, lenalidomide was tested in a phase 2 trial in patients with IPSS low-risk or intermediate-1–risk disease who were red blood cell transfusion-dependent and had a deletion of chromosome 5q31, either alone or in association with other chromosomal abnormalities. Of 148 patients enrolled in this phase 2 study, 67% achieved transfusion independence, with a median time to response of 4.6 weeks. The median increase in hemoglobin was 5.4 g/dL and the median duration of response was >2 years. A major cytogenetic response (ie, elimination of the del(5q) clonal abnormality) occurred in 44% of patients. The major adverse effect was myelosuppression, with grade 3 to 4 neutropenia and thrombocytopenia seen in up to 55% of patients; treatment-emergent cytopenias are associated with a moderately higher likelihood of response. These results led to the approval of lenalidomide by the FDA in 2005 for patients with del(5q) with IPSS low-risk or intermediate-1–risk disease who are red blood cell transfusion-dependent.

A second phase 2 trial of lenalidomide was conducted in patients with the same eligibility who did not have del(5q). In this patient population, responses were less frequent and of shorter duration compared to those in patients with del(5q); 26% of patients became red blood cell transfusion independent, with a median response duration of 41 weeks. Lenalidomide at high doses (>10 mg/d) has some clinical activity in patients with high-risk disease (eg, excess blasts or complex karyotype) or AML, but is not FDA-approved for these indications. Patients with a low platelet count are much less likely to achieve benefit from lenalidomide than those with a platelet count $>50 \times 10^9$/L.

Luspatercept is a TGF-β superfamily ligand-trapping fusion protein consisting of the extracellular domain

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activin receptor IIB (ActRIIB) linked to the human immunoglobulin G1 (IgG1) Fc domain. Luspatercept has been recently FDA-approved in ESA-refractory, transfusion-dependent MDS in patients with IPSS-R very-low- to intermediate-risk myelodysplastic syndromes with ring sideroblasts (MDS-RS) or MDS/MPN-RS-T. ActRIIB is a member of the TGF-β superfamily of proteins, where the downstream SMAD family of transcription factors are important regulators of hematopoiesis. After ligand binding and receptor phosphorylation, the SMAD signaling pathway becomes activated. In conditions of ineffective erythropoiesis such as MDS and β-thalassemia, increased SMAD2–SMAD3 signaling inhibits red cell maturation. Luspatercept binds select transforming growth factor β superfamily ligands to decrease SMAD2 and SMAD3 signaling and enable erythroid maturation by means of late-stage erythroblast differentiation. Luspatercept is administered by subcutaneous injection every 3 weeks. In a randomized, multicenter, double-blind, placebo-controlled trial in 229 patients with transfusion-dependent IPSS-R very-low-, low-, or intermediate-risk MDS with ring sideroblasts, 37.9% patients achieved 8-week transfusion independence (95% confidence-interval [CI]: 30.2, 46.1) compared to 13.2% (95% CI: 6.5, 22.9) in the placebo group, with a treatment difference between groups of 24.6% (95% CI: 14.5, 34.6; $P < 0.0001$.) The most common adverse reactions to luspatercept were fatigue, headache, musculoskeletal pain, arthralgia, dizziness/vertigo, nausea, diarrhea, cough, abdominal pain, dyspnea, and hypersensitivity.

Finally, targeted therapy with inhibitors of isocitrate dehydrogenase 1/2 may have a role in MDS. Mutations in the isocitrate dehydrogenase 1 (IDH1) or 2 (IDH2) genes occur in about 4% to 12% of MDS patients. Clinical trials of IDH1/2 inhibitors in MDS are ongoing, but early clinical trials suggest that MDS patients with IDH1/2 mutations can respond to these inhibitors both as monotherapy and in combination with HMA.

Immunotherapy

An autoreactive T-cell–mediated process suppressing hematopoiesis may contribute to pancytopenia in some patients with MDS. Several studies have demonstrated that treatment approaches analogous to IST of AA may be beneficial in MDS. Therapy with ATG and CsA (as described in the AA section) benefits some patients with lower-risk disease (<10% blasts), especially those who are <60 years of age, lack transfusion dependence, show marrow hypocellularity, and have either a normal karyotype or trisomy 8. Selection of patients most likely to respond to ATG or cyclosporine therapy remains challenging.

KEY POINTS

- Azacitidine has been demonstrated to improve survival by a median of 9 months in patients with higher-risk MDS. Decitabine, another hypomethylating cytosine analog, also produces responses in MDS and delays AML progression. Oral decitabine and cedazuridine provides equivalent pharmacokinetic and safety compared to intravenous decitabine. All 3 drugs are approved by the FDA for the treatment of MDS.
- Azacitidine and decitabine induce DNA hypomethylation through the inhibition of DNMT1, but it is not clear whether this mechanism is responsible for the clinical effects.
- Lenalidomide led to transfusion independence in 67% of lower-risk MDS patients with deletions of chromosome 5q, and some patients also achieved a cytogenetic remission. Lenalidomide also has some effectiveness in anemic patients with lower-risk MDS who lack deletion of chromosome 5q, but the response rate is only 20% to 25%, and responses are not durable.
- Lenalidomide's mechanism of action in MDS is via binding to cereblon and modulation of ubiquitination of casein kinase 1 and alteration of casein kinase's clearance rate. Casein kinase is encoded on chromosome 5q.
- Luspatercept is an TGF-β superfamily ligand trap that improves transfusion independence in ESA-refractory MDS patients and is FDA-approved for use in very-low-risk to intermediate-risk MDS with ring sideroblasts.
- Some patients with hypocellular MDS respond to ATG or cyclosporine/tacrolimus immunotherapy but selecting the most appropriate patients for this therapy remains challenging. Younger patients and those with lower-risk disease (ie, those who are not yet transfusion-dependent or have required transfusions for only a short time) with normal karyotype or trisomy 8 seem most likely to benefit.

Supportive care: transfusions and iron chelation

Despite the availability of several active treatments for MDS, transfusion support remains a mainstay of therapy for many patients. Patients receiving red blood cell transfusions at least once every 8 weeks have a poorer survival than those who do not require regular transfusions, probably because a need for transfusions is a marker of more advanced hematopoietic failure and higher-risk disease. In a number of studies, lower-risk patients with MDS who have ferritin >1000 ng/mL have been shown to experience poorer survival than lower-risk MDS patients with ferritin ≤1000 ng/mL, suggesting that transfusion-related iron overload also might be a contributing factor to poorer outcomes in transfusion-dependent patients. In light of this, RBC transfusions should be minimized and used

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only as necessary for symptomatic anemia or to maintain a safe hemoglobin of 7 to 8 g/dL. Platelet transfusions also may be necessary in some patients with MDS who have bleeding episodes, but the development of alloimmunization is problematic.

Because the correlation between serum ferritin and iron burden is relatively poor and patients receiving transfusions develop iron overload at different rates, newer techniques for noninvasively measuring hepatic iron concentration, such as quantitative (R2*/T2*) magnetic resonance imaging, may be useful in determining which patients are the best candidates for iron chelation. Cardiac T2* magnetic resonance imaging results may also be clinically helpful in determining patients' risk from iron overload, but T2* signals in the heart are rarely abnormal until patients have received at least 80 to 100 units of blood.

Consideration should be given to initiation of iron chelation therapy with parenteral deferoxamine or oral deferasirox in low-risk MDS patients who have a reasonable life expectancy, are red blood cell transfusion-dependent, and have evidence of tissue iron overload. One randomized phase 2 study evaluated the outcomes of deferasirox compared to placebo in patients with low- to intermediate-1–risk MDS and demonstrated that deferasirox prolonged the median event-free survival by about a year. In elderly patients with MDS, a dose of deferasirox high enough to cause a negative iron balance (ie, at least 20 to 30 mg/kg/d) often results in elevated creatinine or intolerable gastrointestinal symptoms. Deferasirox and deferoxamine should be avoided in patients with creatinine clearance less than 40 mL/min. Deferiprone (L1) is widely used for chelation therapy in thalassemia, but a risk of agranulocytosis limits its use in MDS.

Hematopoietic growth factors

Hematopoietic growth factors are an integral part of the treatment of MDS, despite the lack of a specific FDA-approved indication for any of the available agents. ESAs in particular may reduce transfusion requirements by improving hemoglobin levels, and these agents are generally well-tolerated.

Studies with recombinant ESAs (epoetin and darbepoetin) demonstrated erythroid response rates in the range of 20% to 40%. The combination of ESA and G-CSF may be more effective in improving anemia than treatment with ESA alone, especially in patients with MDS-RS. No prospective studies have shown an alteration in survival with ESAs in MDS, although several retrospective studies suggest that ESAs may improve life expectancy and there is no increase in AML progression with ESA use.

An 8- to 12-week trial of an ESA at standard dosing schedules is appropriate for anemic patients with serum erythropoietin levels <200 to 500 U/L. Patients with serum erythropoietin levels >500 U/L respond only rarely to ESA therapy, and patients who have heavy transfusion needs are less likely to respond than those who do not require transfusions.

Both G-CSF (filgrastim, tbo-filgrastim) and granulocyte-macrophage colony-stimulating factor (sargramostim, molgramostim) have been evaluated in patients with MDS and increase the neutrophil count in up to 60% to 90% of patients, which may help some patients who have recurrent infections. Concerns regarding use of G-CSF and risk of leukemic transformation were addressed in a randomized controlled trial of 102 patients with high-risk MDS who were treated with either G-CSF or supportive care. No differences in frequency or time to progression to AML were seen between the 2 groups overall, but survival was shorter in patients with 5% to 19% blasts who received G-CSF.

Thrombopoietin (TPO)-receptor agonists approved for use in immune thrombocytopenia, romiplostim, and eltrombopag have been evaluated in clinical trials for patients with lower-risk MDS and can improve the platelet count in many patients and reduce bleeding events. Patients who are not heavily platelet transfusion-dependent and who have an endogenous TPO level <500 pg/mL are most likely to benefit. However, some patients experience an increase in blood or marrow blast proportion during romiplostim or eltrombopag therapy, which may be because some myeloblasts have functional TPO receptors. In one placebo-controlled study of romiplostim monotherapy, progression to AML was observed in 6% of patients treated with romiplostim, compared to 2.4% with placebo, with the majority of progressions seen among patients who already had excess blasts before treatment When the drug is withdrawn, the blast percentage usually decreases and survival has not been shown to be impacted. When romiplostim was used in pilot studies in combination with azacitidine, decitabine, or lenalidomide, however, an increased rate of progression to AML was not observed. Another concern with TPO agonists is the possibility of development of marrow fibrosis with long-term use, because mice engineered to overexpress TPO develop a myelofibrosis-like picture, but the clinical relevance of this is unclear and, to date, fibrosis in TPO agonist–treated patients with MDS has been rare. Rebound thrombocytopenia can occur with discontinuation of TPO agonists. Thrombocytopenic patients who have bleeding from mucosal surfaces (eg, urinary bladder or gut) may benefit from topical therapy or careful use of the antifibrinolytic agent epsilon aminocaproic acid.

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KEY POINTS

- Transfusion support is an integral part of supportive care for most patients with MDS. Iron chelation may become necessary in carefully selected low-risk patients who are receiving regular red cell transfusions.
- ESAs lead to a red blood cell response in ~20% to 40% of patients; adding G-CSF to ESAs can lead to red blood cell response in ~40% of patients, and responses to combined therapy may be more common among patients with MDS-RS.
- ESAs are less effective in patients with high pretreatment serum erythropoietin levels (≥500 U/L).
- TPO receptor agonists (thrombopoiesis-stimulating agents) can raise the platelet count in some patients with MDS and decrease platelet transfusions and clinically significant bleeding events, but they have been associated with increased blast proportion in some cases and are not FDA-approved for MDS.

Allogeneic HSCT

Although allogeneic HSCT is the only potentially curative therapy in MDS, <10% of patients with MDS currently undergo HSCT because of older age, comorbid conditions, lack of a suitable donor, high cost, patient concern over the risk of transplantation, and failure of clinicians to refer patients who might be transplant candidates to transplant centers. Younger patients (ie, <40 years) without excess blasts at the time of transplant may have a long-term disease-free survival rate exceeding 50% after an HLA-matched HSCT. Patients with high IPSS score or treatment-resistant disease have survival rates <30% after HSCT. Patients with a complex monosomal karyotype, defined as 2 or more autosomal monosomies or 1 monosomy plus additional structural chromosomal abnormalities, are at particularly high risk for poor outcome. When patients with complex karyotype also have a *TP53* mutation, the long-term disease-free survival even with HSCT is <10%.

Allogeneic HSCT should be considered for patients with higher-risk MDS who have an adequately HLA-matched donor and a good performance status. The use of reduced-intensity conditioning regimens may permit allogeneic HSCT in older individuals up to the age of 75.

Given the risks of allogeneic HSCT, defining the optimal time to refer patients for transplantation is an important consideration. Decision analyses have indicated that performing transplantation in patients with lower-risk disease only at the time of disease progression results in greater life expectancy than when HSCT is performed earlier in the disease course. In contrast, patients with higher-risk disease benefit from HSCT near the time of diagnosis. Unfortunately, disease relapse occurs in the majority of high-risk patients after HSCT and thus represents a continuing challenge. Prognostic models to assess HSCT risk have been proposed that stratify by many of these factors. Strategies to reduce relapse rates are being studied and include pre- and posttransplantation interventions with novel therapies. Pretransplantation therapy with HMAs in high-risk patients may be useful to reduce the burden of marrow blasts or if transplant is delayed. Recent publications have shown that genetic profiling pre-HSCT can predict clinical outcomes post-HSCT as well as inform selection of conditioning. *TP53* mutations were associated with shorter survival after transplant, as were *RAS* pathway mutations. There was no benefit to myeloablative regimens over reduced-intensity regimens in patients with *TP53* mutations.

HSCT is the treatment of choice for children and young adults with MDS. It is imperative to perform a diepoxybutane test to exclude FA before performing a transplantation in a child or young adult with apparently de novo MDS, because patients with FA suffer severe toxicity with conventional conditioning regimens and also require close monitoring for nonhematologic tumors after transplantation. Although most patients with FA have dysmorphic features such as short stature and radial ray anomalies, many do not. A bone marrow examination and cytogenetic testing should be performed on any related donor when the recipient is a child or young adult with bone marrow monosomy 7, because there have been instances in which an unsuspected clonal disorder has been detected in the prospective donor. Particular care must be exercised in determining the proper conditioning regimen and best time to perform HSCT in infants and young children because of the toxic effects of radiation on the developing central nervous system and because of differences in drug metabolism compared to older children and adults.

KEY POINTS

- Allogeneic HSCT remains the only curative approach in MDS and is an important consideration for many patients. Cure rates overall are ~30% to 40%.
- Reduced-intensity (nonmyeloablative) conditioning regimens are associated with a lower transplantation-related mortality but higher relapse rate in MDS; overall survival is similar with reduced-intensity and conventional myeloablative conditioning. Reduced-intensity conditioning regimens may permit HSCT to be performed in older patients.

Key Points continue

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- Transplantation at the time of progression for patients with lower-risk disease, and as soon as feasible after the time of diagnosis for patients with higher-risk disease, yields the greatest life expectancy.
- HSCT is the treatment of choice for pediatric MDS; however, donors and recipients must be screened carefully to exclude familial disorders such as FA that would alter the management.

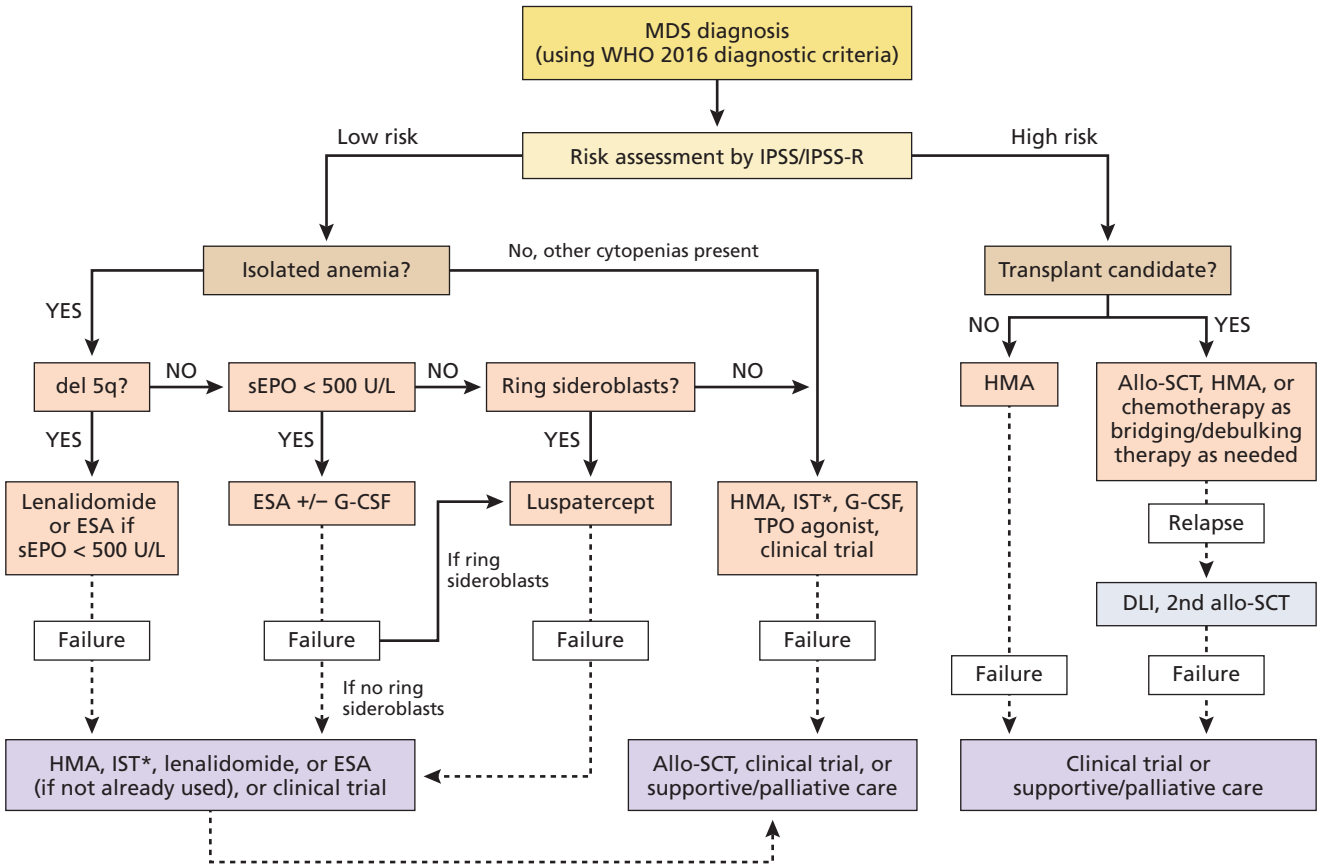
General therapeutic approach

An approach to MDS therapy is outlined in Figure 18-10. All patients should receive supportive care with transfusions and antimicrobial agents as needed. Iron chelation therapy can be considered for selected RBC transfusion-requiring, lower-risk patients with an expected long life expectancy and evidence of transfusional hemosiderosis.

For lower-risk patients (ie, those without excess blasts or an adverse karyotype) in whom the clinical picture is dominated by anemia, the initial therapeutic choice depends on the karyotype and the serum erythropoietin (EPO) level. For patients with del(5q), lenalidomide is an appropriate first choice and is FDA-approved for this indication. For patients without del(5q) but with serum EPO <200 to 500 U/L, ESAs such as epoetin or darbepoetin are recommended. Iron stores should be monitored with ESA therapy and repleted if needed. For low to intermediate-risk patients with ring sideroblasts and transfusion-dependent, ESA-refractory anemia or EPO >200 U/L, luspatercept may improve transfusion needs.

The most appropriate therapy for lower-risk patients with either anemia with serum EPO >500 U/L and without del(5q)/ring sideroblasts, pancytopenia, or a

Figure 18-10 A general approach to MDS therapy, as described in the text. All patients should receive supportive care. Low-intensity therapies are most suited for lower-risk MDS, whereas patients with higher-risk disease should move to allogeneic stem cell transplant, if feasible, or otherwise be considered for azacitidine or decitabine therapy. The presence of ring sideroblasts is defined as ≥15% ring sideroblasts or ≥5% ring sideroblasts if an SF3B1 mutation is present, and with <5% bone marrow blasts. allo-SCT, stem cell transplantation; DLI, donor lymphocyte infusion; HMA, hypomethylating agent (azacitidine or decitabine or decitabine/cedazuridine); sEPO, serum erythropoietin level. Based on National Comprehensive Cancer Network guidelines; see http://www.nccn.org. *Candidates for IST may include: (1) patients who are age 60 years or younger with less than or equal to 5% marrow blasts; (2) patients who have hypocellular marrows; (3) patients with HLA-DR15 positivity; (4) patients with PNH clone positivity; or (5) patients with T-cell clones.



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clinical picture dominated by individual cytopenias other than anemia (ie, neutropenia or thrombocytopenia) is unclear. HMAs can be beneficial in some patients with lower-risk disease, though their effect on survival in this group is unclear. Patients with isolated thrombocytopenia may overlap with immune thrombocytopenia and may benefit from corticosteroids, romiplostim, intravenous gamma globulin, or other immune thrombocytopenia-directed therapies. IST, lenalidomide, supportive care alone, or HSCT are all reasonable choices in the other patient groups, depending on patient-specific factors. Many of the patients in these groups do not truly have "lower-risk" disease—for instance, the population with pancytopenia is enriched for those with *EZH2* mutations, which confers increased risk—and, in the future, molecular profiling may help assign them to a higher-risk group, likely resulting in increased therapy with HMAs or other potentially disease-modifying approaches.

For higher-risk patients, the treatment approach differs depending on whether the patient is a transplant candidate. Higher-risk patients who are HSCT candidates should proceed with definitive HSCT therapy as soon as feasible. HSCT may be preceded by a few treatment cycles of a HMA as a bridging therapy to try to cytoreduce or at least keep the disease stable until a donor is identified, insurance approval is obtained, and pretransplant screening tests are completed. Patients who are not HSCT candidates can be treated with an HMA; some investigators prefer azacitidine over decitabine because of the demonstrated survival advantage in this setting. Oral decitabine and cedazuridine appears equivalent to intravenous decitabine. The addition of venetoclax to HMA may improve response rates in treatment-naïve high-risk patients or recapture response in patients with HMA-refractory disease but is associated with hematologic toxicity.

Once initial therapy fails, no optimal second-line therapy is defined, and the choice depends on clinical circumstances. Supportive care is the default, and clinical trial enrollment is always appropriate, if available.

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